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THE EFFECT OF SOME SUBSTANCES INFLUENCING CELL ACTIVITY UPON THE GROWTH OF THE ROUS No. 1 SARCOMA.

J. G. CARR.

From the Institute of Animal Genetics, University of Edinburgh.

Received for publication July 15, 1942.

A LARGE number of experiments have been reported describing the effect of the administration of substances upon malignant growths. Few workers have utilized the virus-induced avian sarcomata for this type of work, yet in many respects these tumours are the most satisfactory material for such investigations. The Rous tumour originates from the animal's own tissues, and hence iso-antibody effects are eliminated, and it is unlike spontaneous or chemically-induced neoplasms in that the biological and chemical nature of the Rous tumour is the same in each animal. At the end of experiments designed previously to determine the mode of inheritance of the resistance of fowls to inoculation with the Rous No. 1 agent, a number of birds with small tumours whose further progress was very slow were available. These seemed suitable for testing the effect upon the Rous No. 1 sarcoma of various substances which from their known action on cell activity might be expected to have some effect upon the tumour, and the results are reported in this paper.

MATERIAL.

Except where otherwise stated, all birds were from the "Non-Susceptible" strain of the inbred Brown Leghorns maintained at this Institute. At the age of 6 weeks they had been inoculated with the Rous No. 1 agent, the largest dose of about 1000 minimal infective doses being given into the muscles of the right breast, and decimal dilutions of this into the opposite breast, right and left leg respectively. Even the largest doses produced only small tumours, about 0·1 to 5 g. weight. The test was terminated 28 days after inoculation, by which time the tumours had been about the same size for 10 days. Untreated birds showed slow growth or a slow regression of the tumour during the next 14 days. As such changes are not subject to irregular fluctuations, the birds were used within a week of the end of the susceptibility test.

Other tests were performed upon birds from other strains of the Institute Brown Leghorns taken at random, and these will be referred to as "controls."

EXPERIMENTAL.

Experiments with colchicine.

The ability of colchicine to arrest mitosis has led several workers to try its action upon neoplastic growth. An inhibition of the development of the Shope papilloma as a result of administering colchicine has been reported by Peyron and collaborators (1936, 1937). No effect was found on tumours of rats and mice by Brues, Marble and Jackson (1940), even when sublethal doses were employed, and a similar lack of effect is reported by Clearkin (1937), Ludford (1936) and by Poulsson (1935) who used mouse tumours alone. These negative results were obtained with both spontaneous and transplanted tumours. Amoroso (1935) in a preliminary paper stated that regression of mouse tumours (M.63) was obtained after treatment, and Dittmar (1939) claimed a reduction in growth of the Ehrlich mouse sarcoma, while Lits (1936) and Lits, Kirschbaum and Strong (1938) stated that mice bearing a lymphoid leukaemia survived longer if treated with colchicine.

Solutions containing 1 mg. of colchicine were injected into 11 fowls bearing Rous tumours. One bird died, and most of the rest were unwell for about 24 hours, but recovered soon after. Doses of 2 mg. were invariably fatal. In seven of the birds the colchicine had no apparent effect upon the tumour, and in the remaining three, a possible slight stimulation of the tumour was recorded. Microscopical examination of the testes of birds given this amount of colchicine 24 hours previously showed an undoubted arrest of cell division. Administration of this amount of colchicine to birds whose tumours had completely regressed a short time previously did not produce any recurrence of the tumour. The same amount given to four "control" birds with small, non-progressive tumours produced in all cases an apparent stimulation of the tumour.

In spite of the failure of colchicine to arrest the growth of the tumour, an inactivation of some of the virus may have occurred. To test this, 1 mg. of colchicine was injected intravenously into a "control" fowl bearing large tumours produced by an inoculation of agent 29 days previously. The bird reacted badly to the injection, but had almost completely recovered by the next day. It was then killed, and a portion of the largest tumour (which was very haemorrhagic) was processed to recover the virus by the method described by Amies and Carr (1939) (centrifuging cell-free extract at pH 5 on a laboratory centrifuge). Tumours were produced by an amount of this processed material corresponding to 10^{-5} g. of tumour, which is within the limits usually found in the Rous No. 1 tumour (10^{-5} to 10^{-6} g.). No inactivation was therefore demonstrated. In addition, the effect of colchicine *in vitro* upon the Rous No. 1 agent freed from cells was determined. A suspension of concentrated virus was diluted until the amount present per c.c. was equal to the yield from 1 g. of tumour, and two lots of 1 c.c. taken. To one, 0·1 c.c. of a solution of colchicine containing 0·375 mg./c.c. was added, and half an hour later the two solutions were titrated simultaneously upon two groups of three birds, with the result shown in Table I. It is evident that no permanent inactivation of the virus had occurred.

TABLE I.—*Effect of Colchicine on Free Rous Agent.*

Dilution.	Site.	Fowl 212. Control treated.		Fowl 213. Control treated.		Fowl 214. Control treated.	
10^{-3}	Breasts	+	+	+	+	+	+
10^{-5}	Legs	+	+	+	+	+	+
		Fowl 215.		Fowl 216.		Fowl 217.	
10^{-4}	Breasts	+	+	+	+	+	+
10^{-6}	Legs	−	−	+	−	−	+

+ Indicates tumour growth. The size of the tumour on each side was substantially the same.

Finally, three " control " birds were injected with colchicine and later inoculated with Rous No. 1 agent, in order to see if the susceptibility of the cells to the agent was affected by this treatment. After injection of 1 mg. of colchicine, one died within 24 hours, and the others were inoculated with decimal dilutions of a suspension of Rous No. 1 agent of rather weak activity the day after colchicine was given, and three untreated " control " birds were similarly injected with the agent. Tumours grew equally well in all birds.

In addition five birds bearing Rous sarcomas were given colchicine and then killed 1–5 days later, and the tumour examined histologically. An increase in the amount of haemorrhage and leucocytic infiltration was found, and this may have been responsible for at least part of the increase in tumour size sometimes noted after treatment.

Experiments with acenaphthene.

It has been shown (Kostoff, 1937, 1938 ; Levan, 1940) that acenaphthene has an action rather similar to that of colchicine, and is active in certain cases (e.g., in *Colchicum* (Levan)) where colchicine has no effect. Though it is present in the carcinogenic fraction of tar, it is devoid of carcinogenic properties when tested upon mice (Bloch, 1922 ; Twort and Fulton, 1930 ; Kennaway, 1930). Haddow and Robinson (1937) reported that it would slightly inhibit the Walker rat carcinoma 256, and found that it produced inhibition in one out of five spontaneous mouse neoplasms.

Since acenaphthene is only feebly soluble in water, it was dissolved in arachis oil (1 g. in 10 c.c.) by heating in a water-bath, and after cooling in air, 0·5 to 1 c.c. of the resulting fine suspension was injected into the left leg of 24 birds bearing tumours. A fine suspension is necessary in order to produce activity in plants (Kostoff, 1938). The oil alone produced no effect when tested on three tumour-bearing birds. The results were very irregular, seven birds showing no effect, ten showing a regression of the tumours, and seven showed a more or less marked stimulation of the tumours. Some of the birds showing regression were kept for several months, but there was no reappearance of the tumour. In four of the cases in which the tumour was stimulated the largest tumour was not acted upon to an extent which enabled it to maintain its superior size.

*Case history. Fowl G.*558.—Inoculated with Rous No. 1 agent 10.vi.41.

Small tumours appeared in breasts only, and these remained small until 10.vii.41. (Three controls injected with the same material had produced tumours with all dilutions of the agent in two cases, and with the three highest concentrations only in the other bird.) The bird was then given 1 c.c. of the acenaphthene solution into the left leg (site of inoculation of the highest dilution of agent). The tumours began to grow rapidly, and post-mortem on 22.vii.41 the conditions were as shown in Table II.

TABLE II.—*Effect of Acenaphthene on Tumours.*

Agent given 10.vi.41.	Site.	Tumour size.	
		10.vii.41.	Post-mortem 22.vii.41.
Full dose (100–1000 min. infective doses)	R. breast	±	Doubtful trace
1/10 full dose	L. ,,	±	++
1/100 ,,	R. leg	—	++++
1/1000 ,,	L. ,,	—	—

The range of tumour size from least amount visible to maximum is expressed by the series ±, +, ++, +++, ++++.

This type of result appeared irregularly, and neither parentage nor original tumour size seemed to be concerned. The cells of one of these stimulated tumours was transplanted to five " non-susceptible " birds whose tumours had regressed, but growth did not result in three birds, and only a very small tumour appeared in the others. This is similar to the result expected when cells from " controls " are transplanted into these birds, so that an alteration of the tumour to a type which will grow in the " non-susceptible " birds cannot be the cause of the sudden growth. Study of the testes of birds killed a few days after injecting acenaphthene showed that the process of cell division had been interfered with by this substance.

Experiments with 2 : 4-*dinitrophenol.*

It has been shown that 2 : 4-dinitrophenol and related compounds will cause a marked increase in the rate of carbohydrate metabolism of both normal and neoplastic tissues (Dodds and Greville, 1933, 1934) and a marked pyrexia is produced when these chemicals are injected into animals (Tainter and Cutting, 1933). Either effect may be expected to modify the rate of growth of a neoplasm. Vannfält (1936) reported that the substance had no effect upon tar-induced tumours in mice, and Emge, Wulff and Tainter (1933) stated that a transplantable rat tumour continued to grow after treatment, though some destructive changes were found in the body of the tumour. They considered that the surface of the tumour showed an increased growth.

A solution of 2 : 4-dinitrophenol was prepared by dissolving it in a little NaOH solution and neutralizing this with HCl, and then diluting to 1 per cent. strength. This solution was given intravenously. The maximum dose tolerated by the chicks was 0·5 to 1·0 c.c., depending upon the size of the birds. Four birds survived when such cases were given for eight consecutive days. There was no apparent effect upon the progress of the tumour, nor was there any change in the rate of growth after the treatment was discontinued.

Experiments with methylcholanthrene.

For an account of the many studies on the effect of chemical carcinogens on neoplasms, the comprehensive reviews of Cook and Kennaway (1938, 1940) and the papers of Haddow and his collaborators (Haddow, 1935, 1938 ; Haddow and Robinson, 1937, 1939 ; Badger *et al.*, 1942) should be consulted.

A 1 per cent. solution of methylcholanthrene in arachis oil was made in the same way as described for the acenaphthene solution, and 0·5 c.c. injected into the left leg of the following birds : (1) Ten birds of the Non-Susceptible strain, 28 days after the test inoculation of Rous agent ; the tumours of three birds had regressed, the others all bore small tumours. (2) Two Non-Susceptible birds whose tumours had regressed, treated 42 days after the test inoculation. (3) One "Control" bird with small tumours present 43 days after inoculation with Rous agent. (4) Two normal eight-week-old chicks. The latter showed no definite reaction to the inoculation over a period of eight weeks. (Neither has the arachis oil any effect upon Rous-infected birds ; see acenaphthene section.) Among the Rous-infected birds, in one case the tumour showed a marked increase in its growth-rate. This bird, which was one of those of Group (1), had a small tumour in the right breast. Within a few days of the injection of methylcholanthrene the tumour began to grow rapidly, and the bird was killed when moribund 20 days after the injection with a large tumour filling the whole of the right breast. In all other cases in which a tumour was present before inoculation these tumours began to decrease in size, and by the end of the third week they were no longer palpable, nor were any signs of them detected later post-mortem. All birds of the first three groups, including the animal whose tumour was stimulated, developed a very large swelling of the treated leg about 9 days after the injection of the methylcholanthrene. One bird was killed and examined at this time. The swelling involved the whole leg and extended into the lower part of the breasts. Between the skin and muscles was a layer of yellow gelatinous matter. When the leg-muscles were cut, a watery fluid containing numerous fat droplets oozed from them, and compression by forceps expelled a further large amount of similar fluid. A few blood-spots were seen in the muscles, but nothing else of an abnormal character. The swelling slowly subsided in the remaining birds and a few lumps began to appear in various parts of the leg, and by the end of the third week the oedema was no longer apparent. One bird killed on the 17th day after inoculation showed some oedema and a small tumour between the leg muscles. In another bird, killed on the 28th day after inoculation, no oedema was detected, but there was a large tumour in the leg. From about 28 days onwards the size of the tumours in the legs began to decrease, and by the end of 40 days, when the experiment was terminated and all birds killed and examined, only three of the birds had small tumours left. In two of these, tumours were also found in the lower femoral lymph plexus. No tumours were found at any other sites. Histological examination of all tumours found during this experiment was made. In each case the appearance was that of a typical Rous No. 1 sarcoma, though in two cases the number of mitotic figures seemed to be unusually high.

DISCUSSION.

This work was begun in order to determine whether the growth of the virus-induced tumours was more amenable to treatment by chemo-therapy than those in which an active virus cannot be demonstrated. By using the " Non-Susceptible " strain, a series of tumours not subject to marked variation in rate of growth was available, and at the same time the effect of diverse factors controlling the growth in the various birds was as far as possible eliminated. The substances used may have influenced the growth of the tumours in two ways, either by an effect upon the tumour, or by acting upon the mechanism responsible for the inhibition of growth of the tumour in the birds. The cause of this resistance is not yet known, but there is evidence that it is reduced when the growth-rate of the bird decreases, and is increased during laying, so that a failure to supply sufficient nutrition to the tumour may be tentatively assumed to be at least a part of the mechanism. The cause of the resistance sometimes shown by birds not of this strain has not yet been investigated. The occasional stimulation of the tumour sometimes shown during these tests may thus have been due to the action of the materials in checking the growth of the bird, and so rendering more nourishment available for the tumour. In the case of the colchicine tests, the increase in tumour size may also have been due to an increase in the amount of haemorrhage into the tumour, as this seemed to be more extensive in the colchicine-treated birds than in the others. Boyland and Boyland (1940) noted that a similar increase in the amount of haemorrhage was present in mouse tumours after treatment with colchicine.

The most interesting result is that obtained with methylcholanthrene. The regression of the tumours remote from the site of injection of the hydrocarbon is similar to the findings of Haddow and co-workers in their experiments on spontaneous and transplanted mammalian neoplasms. But at the same time it appears that the cells near to the site of injection of the methylcholanthrene are rendered abnormally sensitive to the action of the virus, and so a Rous tumour, apparently induced by the chemical carcinogen, develops at the point of injection. Since Mellanby (1938) has shown that the Rous virus will infect another non-filterable tumour growing in the same bird, the presence of Rous agent in these tumours was to be anticipated, and no attempt was made to pass them by way of cell-free preparations. It may be possible that a similar localization of a pre-existing virus was obtained when McIntosh (1933) obtained tumours which could be transmitted by a cell-free extract, after inoculation of chemical carcinogens into fowls. The failure of some others to confirm this finding could then be explained simply on the assumption that the particular birds used were free from such viruses. Even so, it is interesting to note that such tumours, though not containing an infective virus, have associated with them a " heavy protein " which chemically and physically resembles the Rous virus very closely. In these cases the localization of a " toothless virus " in the sense of Andrewes (1939) may not be an impossible conclusion. This interaction of the virus and chemical carcinogen recalls that found in the case of the tumours produced in rabbits treated with the Shope papilloma and afterwards with tar (Rous and Kidd, 1936). Further investigation of this interaction is likely to be of considerable interest in relation to the study of

carcinogenesis. For such work, of course, the "Non-Susceptible" strain is quite unsuited. Apart from this remarkable interaction it is worth noting that the Rous No. 1 tumour reacts to such diverse pharmacologically active substances as 2 : 4-dinitrophenol, colchicine and methylcholanthrene in the same way as most workers have reported for mammalian neoplasms.

SUMMARY.

No inhibition of the Rous No. 1 tumour was produced by 2 : 4-dinitrophenol.

Colchicine did not prevent the growth of established Rous tumours, nor would it inactivate the agent present in them either *in vivo* or *in vitro*. Normal birds treated with it showed typical development of tumours after infection with the agent. Treatment with acenaphthene produced irregular results, both regression and stimulation of the tumours resulting.

Injection of methylcholanthrene produced a violent local reaction in birds infected with Rous agent, with subsequent regression of established tumours and development of a Rous tumour at the site of injection of the hydrocarbon about three weeks later.

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REFERENCES.

Amies, C. R., and Carr, J. G.—(1939) *J. Path. Bact.*, **49**, 479.
Amoroso, E. C.—(1935) *Nature*, **135**, 266.
Andrewes, C. H.—(1939) *Proc. Roy. Soc. Med.*, **33**, 75.
Badger, G. M., Elson, L. A., Haddow, A., Hewett, C. L., and Robinson, A. M.—(1942) *Proc. Roy. Soc.*, B, **130**, 255.
Bloch, B.—(1922) *Leeuw.-Vereen.*, **1**, 46. Cited by Kennaway.
Boyland, E., and Boyland, M. E.—(1940) *Biochem. J.*, **34**, 280.
Brues, A. M., Marble, B. B., and Jackson, E. B.—(1940) *Amer. J. Cancer*, **38**, 159.
Clearkin, P. A.—(1937) *J. Path. Bact.*, **44**, 469.
Cook, J. W., and Kennaway, E. L.—(1938) *Amer. J. Cancer*, **33**, 50. (1940) *Ibid.*, **39**, 381, 521.
Dittmar, C.—(1939) *Z. Krebsforsch.*, **49**, 515.
Dodds, E. C., and Greville, G. D.—(1933) *Nature*, **132**, 966.—(1934) *Lancet*, i, 398.
Emge, L. A., Wulff, L. M. R., and Tainter, M. C.—(1933) *Proc. Soc. exp. Biol., N.Y.*, **31**, 152.
Haddow, A.—(1935) *Nature*, **136**, 868.—(1938) *J. Path. Bact.*, **47**, 567.
Idem and Robinson, A. M.—(1937) *Proc. Roy. Soc.*, B, **122**, 442.—(1939) *Ibid.*, **127**, 277.
Kennaway, E. L.—(1930) *Biochem. J.*, **24**, 497.
Kostoff, D.—(1937) *C. R. Acad. Sci. U.R.S.S.*, **19**, 197.—(1938) *Nature*, **141**, 1145.—(1938) *Ibid.*, **142**, 753.
Levan, A.—(1940) *Hereditas*, **26**, 262.
Lits, L. J.—(1936) *Arch. int. Méd. exp.*, **11**, 811.

Idem, KIRSCHBAUM, A., AND STRONG, L. C.—(1938) *Proc. Soc. exp. Biol. N.Y.*, **38**, 555.
LUDFORD, R. J.—(1936) *Arch. exp. Zellforsch.*, **18**, 411.
MCINTOSH, J.—(1933) *Brit. J. exp. Path.*, **14**, 422.
MELLANBY, E.—(1938) *J. Path. Bact.*, **47**, 47.
PEYRON, A., POUMEAU-DELILLE, G., AND LAFAY, B.—(1937) *C. R. Soc. Biol., Paris*, **126**, 685.
PEYRON, A., LAFAY, B., AND KOBOZIEFF.—(1936) *Bull. Ass. franç. Cancer*, **25**, 874.
POULSSON, K. T.—(1935) *Norsk Mag. Lægevidensk*, **96**, 735.
ROUS, P., AND KIDD, J. G.—(1936) *Science*, **83**, 468.
TAINTER, M. L., AND CUTTING, W. C.—(1933) *J. Pharmacol.*, **48**, 410.
TWORT, C. C., AND FULTON, J. D.—(1930) *J. Path. Bact.*, **33**, 119.
VANNFÄLT, K. A.—(1936) *Uppsala LäkFören. Forh.*, **42**, 55.

STUDIES ON THE EFFECT OF CARCINOGENIC HYDROCARBONS ON THE PRODUCTION OF ANTIHORMONES.

C. HOCH-LIGETI.

From the Chester Beatty Research Institute, the Royal Cancer Hospital (Free), London, S.W. 3.

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GORDON demonstrated in 1937 that splenectomized rats respond with a longer and more pronounced increase in the weights of the ovaries to the administration of chorionic urinary gonadotropin. He suggests that this effect may be taken as evidence for a connection between the reticulo-endothelial system (R.E.S.) and the production of antihormones, and showed further that the injection of trypanblue, which is taken up by the cells of the R.E.S., increases the effect of splenectomy. In 1940 Emery and his associates injected splenectomized rats, 30 days old, with different gonadotropic preparations and, considering only the effect on the 30th day after the treatment started, failed to show any significant differences in the mean of the weights of the ovaries and uteri between splenectomized and control rats. But as Gordon investigated the whole course of the response in rats 21 to 26 days old, and Emery made his observations at an arbitrary endpoint in somewhat older rats, where the activity of the animals' own ovaries may interfere with the effect of the injected hormones, the comparison of the two experiments is difficult.

The role of the R.E.S. in the development of malignant disease has been investigated in several ways. The administration of carcinogenic hydrocarbons produces alterations in the Congo red index in rabbits (Csato *et al.*, 1939; Hoch-Ligeti, 1940), and a decrease in the size of the spleen and changes in the histological appearance of the lymphatic system in mice (Parsons, 1938; Hoch-Ligeti, 1941*a*). The following experiments represent an attempt to

TABLE I.

	Date.	Strain.	Number of animals receiving—					Without treatment.
			Hormone.	Hormone + methyl-cholanthrene.	Hormone + anthracene.	Methyl-cholanthrene.	Anthracene.	
Series 1	3.iv.41 to 12.v.41	Black-hooded	20	20	—	—	—	—
,, 2	14.v.41 to 20.vi.41	Albinos	20	20	20	—	—	—
,, 3	13.ix.41 to 16.x.41	Black-hooded	20	20	—	20	—	—
,, 4	20.x.41 to 25.xi.41	Albinos	30	30	—	—	—	—
,, 5	14.xi.41 to	Black-hooded	20	20	—	—	—	10
,, 6	15.xii.41	Albinos	20	20	—	—	—	10
,, 7	11.ii.42 to	Black-hooded	20	—	20	—	—	—
,, 8	17.iii.42	,,	—	—	—	20	20	—

TABLE II.—*Weight of the Ovaries of Black-hooded Rats.*

Day of experiment.	Treated with— Hormone. Number of rats.	Range.	Mean.	Hormone + methylcholanthrene. Number of rats.	Range.	Mean.	Treated with— Hormone. Number of rats.	Range.	Mean.	Hormone + anthracene. Number of rats.	Range.	Mean.
0	3	10–23·6	15·3	3	13·4–18·8	16·2	1	11·2	11·2	1	12·4	12·4
3	3	23·3–36·5	23·4	3	35·0–40·8	37·0	1	44·0	44·0	1	40·2	40·2
6	4	46·3–85·5	63·9	4	44·6–85·5	58·3	2	37·5–62·1	49·8	2	47·6–87·0	67·3
9	4	58·4–83·2	71·6	5	57·2–83·9	71·5	2	78·5–94·6	86·5	2	78·1–83·5	80·8
12	5	57·2–76·4	67·5	5	42·0–92·4	68·0	2	56·3–59·6	57·9	2	72·6–13·4	73·0
	Sum of means		241·7			251·0			249·4			273·7
15	5	39·5–119·1	70·7	5	64·8–103·0	83·7	2	69·5–96·7	83·1	2	86·3–118·2	102·3
18	5	16·9– 74·5	48·5	5	62·5–238·7	133·0	2	43·4–71·7	57·5	2	29·9– 57·6	43·7
21	5	24·0– 67·4	37·3	6	95·9–131·4	103·0	2	57·9–95·8	70·9	2	57·0–191·6	124·3
24	5	27·1– 52·6	44·0	6	43·1–227·1	96·5						
27	5	29·2– 56·3	52·0	5	72·2–163·2	112·0	2	41·2–55·6	48·4	2	19·7– 99·9	59·8
30	5	33·8–146·9	69·5	5	64·9–124·1	96·3	2	29·2–95·8	62·6	2	25·3– 37·2	31·3
	Sum of means		322·0			624·5			322·5			361·4

The rats compared in the columns Hormone and Hormone + Methylcholanthrene, and Hormone and Hormone + Anthracene, respectively, and killed on the same day, were litter mates.

TABLE III.—*Weight of Uteri of Black-hooded Rats.*

Day of experiment.	Treated with— Hormone. Number of rats.	Range.	Mean.	Hormone + methylcholanthrene. Number of rats.	Range.	Mean.	Treated with— Hormone. Number of rats.	Range.	Mean.	Hormone + anthracene. Number of rats.	Range.	Mean.
0	1	45·6	45·6	1	43·7	43·7	1	21·5	21·5	1	18·6	18·6
3	2	110·0–110·4	110·2	2	125·9–203·1	164·5	1	80·5	80·5	1	68·4	68·4
6	2	89·6–186·8	143·2	3	103·5–214·7	159·1	2	161·0–179·0	170·0	2	104·1–159·4	131·7
9	3	188·2–253·3	217·0	4	212·7–332·7	273·3	2	176·8–200·1	188·4	2	179·9–222·8	201·3
12	4	228·4–365·5	288·0	4	199·2–290·0	240·0	2	240·5–330·3	285·4	2	250·2–255·2	252·7
		Sum of means	804·0			880·6			745·8			672·7
15	4	207·5–345·5	260·1	4	341·6–458·0	400·8	2	219·1–266·7	243·0	2	274·5–321·9	298·2
18	4	117·8–358·1	214·8	5	273·9–405·5	331·6	2	398·5–299·8	299·1	2	334·2–425·9	380·0
21	4	80·9–285·9	181·3	4	99·7–488·3	238·2	2	335·9–475·7	405·8	2	349·0–417·8	383·4
24	3	125·7–380·2	202·9	4	152·3–769·3*	511·3						
27	3	95·7–320·0	210·5	3	156·9–412·3	323·6	2	182·7–229·3	206·0	2	106·0–389·4	247·7
30	3	154·9–702·2*	486·9	3	149·0–321·8	252·5	2	97·8–329·3	213·5	2	181·0–238·3	209·6
		Sum of means	1556·5			2058·0			1367·4			1518·9

The rats compared in the columns Hormone and Hormone + Methylcholanthrene, and Hormone and Hormone + Anthracene, respectively, and killed on the same day, were litter mates.

* Oestral uterus distended with fluid.

show whether an influence of carcinogenic hydrocarbons on the production of antihormones could be demonstrated. The general procedure of Gordon was followed, substituting for splenectomy the administration of the carcinogenic hydrocarbon methylcholanthrene; the effect of this compound on the production of antihormones to gonadotropin from the urine of pregnant women was investigated.

EXPERIMENTAL.

Four hundred female rats, 21–26 days old at the beginning of the experiment, of two strains, black-hooded and albino, were used and were divided into 8 series (Table I). Those compared on the same dates were litter mates, and were kept in the same cage and received the same food.

Methylcholanthrene (0·5 ml. of a 0·05 per cent. colloid [= 250γ] in 1 per cent. gelatine) was injected subcutaneously into the left groin on the 1st, 8th and 22nd day and later at intervals of 2 to 3 weeks. Each animal received daily injections in the skin of the neck of 10 units of a gonadotropic preparation from urine of pregnant women ("Pregnyl") which contained one I.U. in 7 gamma in one, and in 13 gamma in another batch. Groups of animals were killed every third day for a period of 30 days, and later at longer intervals. The ovaries, uterus, adrenals, thymus, spleen, liver and kidneys were removed and weighed at once, while the pituitaries were weighed after 24 hours' fixation in formol-saline. This communication deals with the effects on the genital tract only. The results obtained with the two strains of rats are considered separately.

RESULTS.

Effect of Hydrocarbons on the Production of Antihormones in Black-hooded Rats.

(a) *Methylcholanthrene.*

The weight of the ovary in all rats receiving gonadotropic hormone increased until the 12th day; after this, the ovaries of animals receiving hormone only decreased in weight, while in those treated with methylcholanthrene also a further increase took place. From the 15th to the 30th day the ovaries from animals treated with hormone and methylcholanthrene were heavier than in those treated with hormone only, in several instances three to four times as heavy. The experiment was repeated three times with the same result. Table II shows the range of variations in the ovary weights, and the means obtained from all animals killed on the same day, all three series being taken together. The sums of the mean weights of all ovaries up to the 12th day are very close—241·7 and 251·0; those of the second period are 322·0 and 624·5 respectively.

(b) *Anthracene.*

The shape of the curve of ovary weights of the 20 animals treated with hormone and anthracene did not differ from that given by the animals receiving hormone only (Fig. 1), except that one rat (21st day) had an unusually high weight of the ovaries, which is obvious in the figure. The sum of the means is

249·4 and 273·7 until the 12th day, and 322·5 and 361·4 in the second period, respectively.

The weights of the ovaries of rats receiving methylcholanthrene only varied between 10 and 49·8 mg. during the experimental period, and of those treated with anthracene between 9 and 49·1 mg. and of the untreated between 11·2 and 32·7 mg., but this difference is not significant when the two series are pooled.

In all animals treated with gonadotropic hormone the opening of the vagina occurred 96 hours after the first injection. The increase in the weight of the uteri in immature animals follows closely that of the ovaries, but the relation becomes uncertain after the 42nd day of life, when natural oestrus may occur. The uterine weights decreased in hormone-treated animals after the 12th day of the experiment, and increased further when methylcholanthrene was given also (Table III). The weights of the uteri in animals receiving methylcholanthrene only show, in one series, an increase from the 9th day of the experiment compared with untreated controls ; in a second series this phenomenon could not be observed.

Histological examination of the ovaries suggests that the increase in weight is caused mainly by an increase of the corpora lutea and of the interstitial tissue ; the latter is often luteinised. The rats receiving methylcholanthrene and hormone show a heavy luteinisation generally at an earlier date, and the number of the ripening follicles in these ovaries is correspondingly smaller. In the later part of the experiment fibrous bodies and cysts lined with fibrous tissue appear. Similar changes have been described by prolonged treatment with gonadotropic hormone by Fluhmann (1939).

The uteri show usually the progestational phase ; only a few oestral uteri distended with secretion were found after the 21st day of the experiment. In several instances a squamous metaplasia of the uterine epithelium could be demonstrated as early as the 15th day. Similar changes of the uterine epithelium under treatment with chorionic gonadotropin were described by Fluhmann. The metaplasia appeared in his cases after about 200 days of treatment. Probably the earlier onset of metaplasia is connected with the administration of carcinogenic hydrocarbon. All untreated animals had infantile uteri during the whole experiment.

Effect of Methylcholanthrene on the Production of Antihormones in Albino Rats.

In the three series of experiments on albino rats the weight of the ovaries showed a peak on the 18th day (Fig. 2 and Table IV). The curves show a steady decrease after the 18th day ; the level, however, does not reach that of the black-hooded rats. The decrease of the ovary weight is similar in the groups receiving hormone, and hormone and methylcholanthrene, but the evaluation of the differences in the change of weight of the ovaries between the two groups is difficult on account of wide individual variations. Nevertheless the means of the ovary weights were somewhat greater in the latter

group from the 21st day onwards than in the former, but that of individual animals is often smaller.

The same shape of curve was obtained with rats receiving hormone and

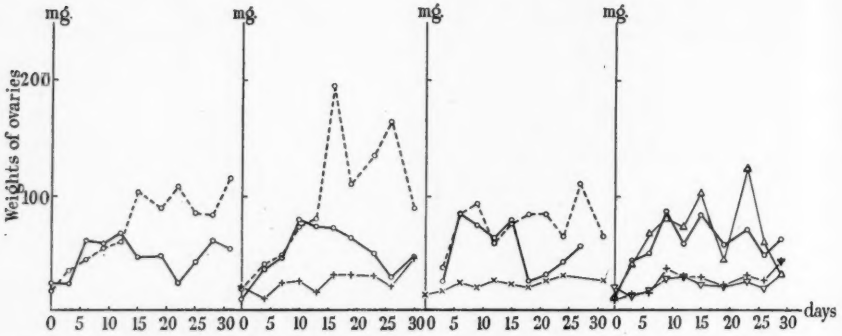

FIG. 1.—Effect of hydrocarbons on the production of anti-gonadotropic hormone in black-hooded rats. The points represent the weights of ovaries, in most cases the mean from two or three rats, in a few cases the weight from a single rat.

—o— Hormone.
– – o – – Hormone + methylcholanthrene.
— + – – Methylcholanthrene.
– – × – – Without treatment.
—Δ— Hormone + anthracene.
—▽— Anthracene.

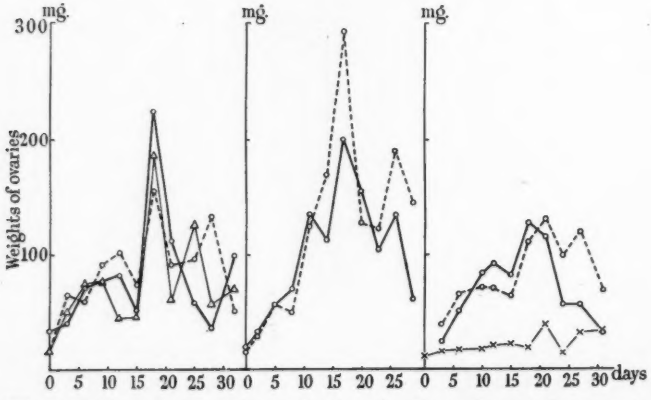

FIG. 2.—Effect of hydrocarbons on the production of anti-gonadotropic hormone in albino rats. The points represent the weights of ovaries, in most cases the mean from two or three rats, in a few cases the weight from a single rat.
See Fig. 1 for explanation of signs.

anthracene. The weights of the ovaries of untreated controls were between 11·2 and 31·0 mg. The changes in uterine weights ran parallel to those of the ovaries (Table V).

The histological investigation of the ovaries and the uteri revealed the same general features as in the black-hooded rats. Corresponding to the smaller

TABLE IV.—*Weight of the Ovaries of Albino Rats.*

Day of experiment.	Treated with— Hormone. Number of rats.	Range.	Mean.	Hormone + methylcholanthrene. Number of rats.	Range.	Mean.	Hormone + anthracene. Number of rats.	Range.	Mean.
0	3	11·2– 33·4	21·3	3	11·2– 15·9	13·7	1	17·0	17·0
3	5	24·1– 40·6	34·4	5	27·8– 58·5	33·0	2	48·7– 54·6	51·0
6	5	50·7– 86·2	60·7	5	53·6– 62·7	65·3	2	60·2– 91·2	75·7
9	4	62·5– 84·7	75·5	4	47·4– 91·5	66·7	1	76·0	76·0
12	6	69·9–135·1	106·0	6	48·5–152·0	98·2	2	34·7– 57·3	45·0
15	7	40·7–156·2	87·5	7	39·4–233·1	122·2	2	34·0– 59·4	46·7
		Sum of means	385·4			399·1			311·4
18	5	74·6–250·9	195·0	5	110·5–312·3	200·5	2	154·4–216·8	185·6
21	6	93·2–168·2	127·2	6	48·5–210·4	116·0	2	40·5– 82·5	61·5
24	7	45·6–140·9	84·5	7	33·5–237·5	104·2	2	79·9–170·1	125·1
27	4	36·3–156·0	90·0	4	119·1–215·5	157·2	1	57·5	57·5
30	5	32·3–129·4	70·5	5	50·2–160·6	116·0	2	61·6– 76·7	70·6
		Sum of means	567·2			693·9			500·3

TABLE V.—*Weight of Uteri of Albino Rats.*

Day of experiment.	Treated with— Hormone. Number of rats.	Range.	Mean.	Hormone + methylcholanthrene. Number of rats.	Range.	Mean.	Hormone + anthracene. Number of rats.	Range.	Mean.
0	2	27·2–123·6	75·4	2	30·7–101·2	65·9	1	123·0	123·0
3	5	62·8–118·3	98·7	5	51·6–201·7	152·6	2	161·6–136·0	148·8
6	5	120·1–258·8	164·4	5	135·9–201·4	176·6	2	224·8–256·0	240·4
9	4	191·7–268·4	228·8	4	221·6–275·4	244·3	1	221·3	221·3
12	6	229·3–363·7	320·0	6	193·9–400·0	262·9	2	291·9–602·7	447·3
15	7	312·4–568·4	382·0	7	256·3–576·6	356·0	2	312·7–341·2	327·0
		Sum of means	1269·3			1258·3			1507·8
18	5	246·9–766·4*	426·1	5	261·6–652·4	468·9	2	381·8–633·0	507·4
21	6	365·9–552·9	376·1	6	207·5–649·8	389·9	2	243·6–607·7	425·6
24	7	118·7–549·6	338·0	7	139·1–652·4	418·0	2	207·9–635·0	421·4
27	4	152·9–493·3	322·5	4	280·4–552·2	451·1	1	105·7	105·7
30	5	114·2–529·3	327·0	5	143·1–814·4*	403·0	2	132·5–357·6	245·0
		Sum of means	1789·7			2130·9			1705·1

* Oestral uterus distended with fluid.

differences in the weight of the ovaries, the differences in the luteinisation were less pronounced. The untreated controls had infantile uteri, except for two, which reached spontaneous oestrus after the 21st day of the experiment.

DISCUSSION.

In a previous experiment on the influence of carcinogenic hydrocarbons on the production of antibodies a similarity between the effect of methylcholanthrene and splenectomy was found (Hoch-Ligeti, 1941*b*). Gordon (1937) claimed that splenectomy influences the production of antihormones. In order to examine further the action of carcinogenic hydrocarbons their effect on the production of antihormones was studied. A remarkable difference in the response to prolonged daily injections of urinary gonadotropic hormone was established in two different strains, black-hooded and albino rats. In the black-hooded rats the result of injection of carcinogenic hydrocarbon corresponded to that of splenectomy in Gordon's experiment; the decline in the weight of the ovaries after the 15th day of treatment with chorionic gonadotropin was prevented. In albino rats, however, a clear-cut result was not obtained. Gordon states that he used black-hooded rats; Emery, in his paper, questioning the results of Gordon, does not mention the strain of animals employed. The differences in the results could be explained if he used albinos.

Differences in the reactivity of various strains of rat to pituitary hormones were observed by Fraenkel-Conrat *et al.* (1940), working on the effect of pituitary interstitial cell-stimulating hormone. This hormone failed to cause an augmentation in ovarian weight in combination with follicle-stimulating hormone, or an increase in the weight of the male accessory organs when applied to the Long-Evans strain, but caused a great increase in the weight of the seminal vesicle when applied to the Harvard strain, while preparations found to be rich in luteinizing hormone when tested on the Harvard strain failed to affect the Long-Evans strain.

In the present experiment the reaction of the ovaries to the same amount of urinary chorionic gonadotropic hormone was greater in the albino than in the black-hooded rats. During the 30 days of the experiment none of the black-hooded treated with hormone alone reached 150 mg.; four of this strain receiving hormone and methylcholanthrene were over 150 mg. and none over 250 mg. In the albinos seven animals treated with hormone and eleven with hormone and methylcholanthrene were over 150 mg., and one with hormone and two with hormone and methylcholanthrene were over 250 mg.

The increase in the weight of the ovaries in the third experiment with each strain was lower than in the two previous ones, but all the general features of the curves were maintained. All known conditions of this experiment were the same as previously except in a change in the diet; the animals in this series did not receive cabbage. The vitamin A content in the liver of these animals was lower than in the animals in the previous experiment; thus it seems possible that this change may influence the response to gonadotropic hormone in rats.

SUMMARY.

The influence of methylcholanthrene on the production of antihormones to chorionic gonadotropin in two strains of rats was studied.

Black-hooded rats produced antihormones from the 15th day of hormone treatment, but when methylcholanthrene was given also the weights of the ovaries increased further, and were in the second 15 days of the experiment on an average twice as heavy as the ovaries of animals receiving hormone only ; in some comparable groups of litter mates this difference was as much as three- or fourfold.

In albino rats the production of antihormones was less pronounced, and the effect of methylcholanthrene doubtful.

The animals treated with anthracene and gonadotropic hormone showed no differences compared with those receiving hormone only. Methylcholanthrene or anthracene alone has no significant effect on the weight of the ovaries.

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REFERENCES.

Csato, T., Wetzler-Ligeti, C., and Wiesner, B. P.—(1939) *Amer. J. Cancer*, **36**, 257.

Emery, F. E., Bugelski, T. J., and Schwabe, E. L.—(1940) *J. Endocrinology*, **26**, 167.

Fluhmann, C. H.—(1939) *Ibid.*, **25**, 193.

Fraenkel-Conrat, H., Hao Li, C., Simpson, M. E., and Evans, H. M.—(1940) *Ibid.*, **27**, 793.

Gordon, A. S.—(1937) *Cold Spring Harbour Symp.*, **5**, 419.

Hoch-Ligeti, C.—(1940) *Amer. J. Cancer*, **39**, 365.—(1941*a*) *Cancer Research*, **1**, 484.—(1941*b*) *Brit. J. exp. Path.*, **22**, 233.

Parsons, L. D.—(1938) *J. Path. Bact.*, **47**, 501.

THE PRODUCTION OF HYPERTENSION IN RABBITS BY THE INTRARENAL INJECTION OF KIESELGUHR WHITE.

B. G. MAEGRAITH AND F. J. MACLEAN.

From the Sir William Dunn School of Pathology, Oxford.

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IT has been shown by many workers that infarction of the kidney by ligation of some of the branches of the renal artery is followed by an elevation of arterial blood pressure (Janeway, 1909*b*; Pilcher, 1913; Cash, 1924; Mark, 1928; Mark and Giesendorfer, 1930; Friedman and Wachsmuth, 1930; Ferris and Hynes, 1931; Wood and Cash, 1936). However, infarction by multiple emboli, liquid paraffin, insoluble Berlin blue, charcoal, and laked red blood cells, has not in the past caused hypertension (Senator, 1911; Cash, 1924; Apfelbach and Jensen, 1931; Scarff and McGeorge, 1937). The results recorded here were obtained by the successful application of a method involving the injection of Kieselguhr White (Maegraith and McLean, 1938), the particles of which are small enough to enter a capillary, although sufficiently long and irregular to be caught up in the bends of the glomerular capillary loops.

I. THE PRODUCTION OF HYPERTENSION.

EXPERIMENTAL.

Technique of injection of Kieselguhr White.

One hundred c.c. of 0·89 per cent. saline solution was brought to the boiling point in a beaker and 1·0 g. powdered gum arabic added and stirred until dissolved. The solution on cooling was made up to 100 c.c. by the addition of distilled water and 1·0 g. well ground Kieselguhr White added and vigorously shaken until suspended. The suspension was equally divided into six test-tubes, which were stopped with cotton-wool and left in a water bath at 56° C. for 24 hours. Before use each tube was shaken and the supernatant suspension was injected as soon as the heavier particles had settled.

Operations were performed under open ether anaesthesia. The abdomen was opened along the line of the linea alba and coils of intestine withdrawn and wrapped in warm moist swabs. The renal artery was exposed and separated from the accompanying nervous tissue. A cotton thread was passed beneath the vessel at a point about 1·5 cm. from the kidney and the ends secured by artery forceps. When the injection was being made, gentle traction was exerted on the artery by pulling on the thread until the vessel was occluded

and the needle was inserted immediately distal to the thread. The suspension was injected at a rate of 1·0 c.c. every 15 seconds, and during the procedure the kidney colour turned to a uniform dirty grey. The needle was withdrawn and a hot moist swab applied to the point of puncture as the traction on the thread was gradually relaxed and the blood permitted to return to the kidney. Normal colour was usually restored to the organ in 1 to 3 minutes. Bleeding could generally be controlled by gentle compression with a swab, but occasionally it was necessary to place a piece of omentum over the site of the puncture. If this failed, the blood-flow in the renal artery was controlled by gentle traction on the thread. When the bleeding had been stopped the intestines were replaced and the abdominal muscle sewn with continuous catgut sutures and the skin with horsehair. The animals were placed on sterile cloth in clean bins in a warm observation room for one to two days following the operation, and later returned to a heated animal house.

Blood-pressure readings.

Pressures were estimated by the carotid loop technique (van Leersum, 1911), using a simple compression cuff and Tycos spring manometer. Blood pressures were recorded by the same observer throughout the experiments in a quiet room set aside for the purpose.

Diet.

The standard diet for all animals in these experiments was 350 g. of mangolds and 60 g. of bran daily, the mangolds being varied once a week by equivalent amounts of greens.

RESULTS.

A. *Blood-pressure changes following the injection of Kieselguhr White.*

1. *Unilateral injections.*—Unilateral injections of Kieselguhr White were made into 23 animals, the dose varying from 3 to 15 c.c. of suspension. Nineteen rabbits exhibited prolonged rises of blood pressure. In some the pressure rose at once and stayed permanently high. In most others the initial rise was succeeded by a fall, followed in a few days by a rise to a permanent high level (Maegraith and MacLean, 1938). In one case the pressure fell to normal before the second rise commenced; in other cases the second rise occurred before the falling pressure had reached the pre-operative level, producing a notched pressure curve. A characteristic blood-pressure curve is shown in Fig. 1.

The hypertension persisted for periods of up to seven months, and in no case did the pressure fall to normal levels without further operative interference, such as nephrectomy or section of the renal nerves. Table I shows the normal pressures and the duration and range of hypertension in 18 of these animals. It should be noted that the hypertensive pressures varied over a much wider range than the normal pressures, and that there was a great difference between the various animals in the extent of the rise. The degree of hypertension was not dependent on the amount of Kieselguhr suspension injected.

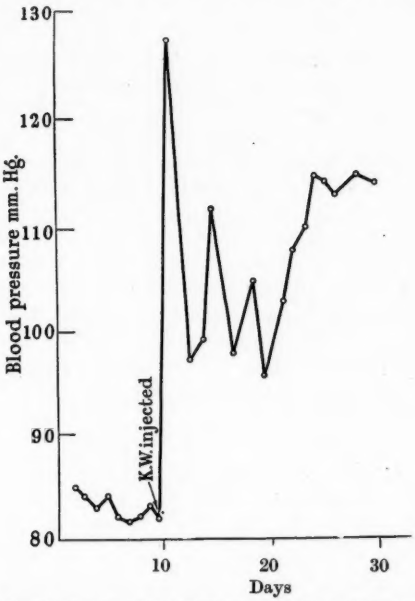


FIG. 1.—Characteristic blood-pressure rise following injection of Kieselguhr suspension into the renal artery. Note the initial spike rise and the subsequent slower rise of pressure.

TABLE I.—*Unilateral Injection of Kieselguhr.*

Normal pressures, and duration and range of hypertensive pressures following unilateral intrarenal injection of Kieselguhr White.

Serial No. of rabbit.	Range of normal pressures (mm. Hg).	Range of hypertensive pressures (mm. Hg).	Duration of rise (days).
1	77–82	95–101	61
2	71–72	82–94	62
3	91–97	109–121	241
4	86–90	115–153	121
5	69–74	80–107	52
6	82–91	98–123	31
7	76–79	88–107	27
8	82–86	114–122	131
9	76–80	88–98	147
10	81–85	102–110	136
11	77–88	97–111	129
12	96–99	106–148	137
13	76–79	103–111	20
14	68–70	94–134	28
15	84–90	125–156	27
16	76–80	104–121	30
17	102–108	126–135	25
18	81–85	102–109	71

2. *Injections into unilaterally nephrectomized animals.*—The injections were made into the main artery, one branch being occluded during the infection by traction on a cotton thread. This procedure was abandoned, as it was found that in such animals damage which was sufficient to raise the blood pressure was enough to kill the animal.

3. *Bilateral injections.*—Five animals of the first series which had had injections into one renal artery later received injections into the other renal artery. Three died within a week from renal insufficiency, and as the already existing hypertension was unaffected in the other two animals, bilateral injections were discontinued.

4. *Control experiments.*—No rise of blood pressure occurred as a result of injection of Kieselguhr into the splenic artery. Physiological saline injected into the renal artery did not cause a prolonged increase in systemic pressure, even when the injection was made so forcibly as to destroy the normal structure of the kidney with ultimate replacement by fibrous tissue.

B. *Urinary changes following Kieselguhr White injection.*

1. *Volume.*—The effect of the injections on the urine volume was studied in ten animals. In seven there was a temporary decrease in output, in two an increase. In two animals after bilateral injection there was a temporary decrease. The periods of decreased urinary volume were accompanied by increased specific gravity, and vice versa.

2. *Sediment.*—Red blood cells, epithelial casts and a few polymorphonuclear leucocytes appeared in the urinary sediment in the acute stages following Kieselguhr injection, disappearing within 7 to 10 days.

3. *Urinary nitrogen.*—Daily determinations of total nitrogen and urea nitrogen were made on the urine samples of seven rabbits over periods of 47 to 104 days, using the method of Conway and Byrne (1933) and Conway (1933, 1934, 1935*b*). In five animals surviving operation long enough to be studied, no constant change was observed in either the total nitrogen or urea nitrogen. However, a comparison of the figures for total nitrogen and urea nitrogen disclosed one important point, viz. following Kieselguhr injection both the relative and absolute amounts of non-urea nitrogen in the urine (calculated as the difference between total nitrogen and urea nitrogen) increased for several days, corresponding to the period of decreased volume and increased specific gravity. During this period the tests for blood and albumen were positive and red blood cells and casts were found in the urine.

C. *Blood changes following Kieselguhr White injection.*

1. *Total blood nitrogen.*—No significant variation from normal levels occurred after injection of Kieselguhr, except in one animal, which exhibited a considerable fall in total blood N during the 8 days it survived injection.

2. *Blood urea nitrogen.*—Every Kieselguhr injection gave rise to an increase in the blood urea nitrogen to a level well above the highest pre-operative reading, and, in two animals which died as a result of injection, the blood urea N rose to great heights before death. In all the other animals the blood urea N returned to normal by the 3rd to the 14th day after injection. In

some cases, however, the post-operative readings were all near the upper limit of the pre-operative range, and in one animal which had had both kidneys injected the normal level was sometimes exceeded even 7 weeks after injection. Removal of the injected kidney in two animals caused small post-operative rises lasting only 1 day. Unilateral nephrectomy in two normal animals caused a rise in blood urea N lasting 2 and 5 days respectively and reaching over 60 mg. per 100 c.c. in both cases.

3. *Blood chloride.*—The technique of chloride estimation was a modification of the method of Conway (1935*a*). The serum chloride of eight normal rabbits varied between 401 and 464 mg. per 100 c.c. No significant variation was effected by injection of Kieselguhr.

TABLE II.—*Blood Urea Changes following Unilateral Kieselguhr Injection.*

Rabbit serial No.	Normal range (mg./100 c.c).	Highest point of rise (mg./100 c.c).	Duration of rise (days).
3	14·1–17·7	49·1	7
4	10·1–19·2	28·6	2
5	9·9–16·1	57·4	7
6	4·8–14·2	31·7	13
7	10·5–19·7	30·7	4
8	10·1–20·1	28·9	7
9	10·4–15·7	41·8	3
10	10·1–14·5	37·9	7
11	11·7–13·8	21·1	2
12	14·4–17·5	67·1	4
13	14·7–15·7	44·4	5
20	11·9–18·3	142·2	1
21	10·6–16·8	31·9	2
21 Other kidney injected	Same	158·8	3

Note.—Rabbits 20 and 21 died.

D. *Changes in the vascular system.*

Except for one case of old pericarditis (Rabbit 2132), no gross lesions were observed in the heart. The aortas were studied in 18 animals exhibiting sustained rises in blood pressure following Kieselguhr injection. Eight of these rabbits showed pathological changes in the aorta, varying from a single small lesion to numerous large plaques. The common site was the ascending aorta and the arch, but in some cases the lesions were scattered through the entire thoracic aorta and a few were found in the abdominal aorta. The histological appearance of the lesions was described by Maegraith and Carleton (1939).

E. *Renal changes following Kieselguhr injection.*

Macroscopic appearances.—With one exception Kieselguhr injection was followed by a reduction in the length of the injected kidney, accompanied by

an increase in the length of the other of up to 4 mm. Unilateral nephrectomy also resulted in hypertrophy of the remaining kidney.

Some kidneys appeared almost normal, even some time after the injection, presenting smooth external surfaces and easily stripped capsules. In other cases the surface of the kidney was mottled and in the later stages became irregular, with pale depressed areas interspersed with patches of apparently normal tissue. The capsule stripped readily over the normal areas, but was adherent over the pitted areas. Examples of such kidneys are shown in Fig. 2, which also illustrates the reduction in size of the affected organ. Such kidneys cut with difficulty, the cut surface presenting patches of normal renal tissue interspersed with areas of necrosis or fibrosis, with calcification in the later stages. This type of kidney and the type described previously were always associated with hypertension, which was usually of greater degree in those animals with kidneys showing least evidence of fibrosis and necrosis of a localized nature. Kidneys which appeared completely necrotic were never associated with hypertension.

Histological appearances.—Immediate effects: Serial sections of kidneys of animals killed within 10 minutes to a few hours after injection of Kieselguhr show the distribution of the particles to be mainly cortical, the polar regions being most affected. The afferent vessels to the glomeruli, and in many cases the glomerular capillaries, are filled with Kieselguhr to the complete exclusion of blood cells. Similar embolism of many of the intertubular vessels can also often be seen in the medulla. The vessels which are not filled with Kieselguhr particles are dilated and filled with blood cells, this reaction being especially notable in the glomerular capillaries. No plugging of the larger vessels has been observed. In the early stages after injection the histological picture thus shows clearly that the injected particles embolize the smaller kidney vessels, especially in the cortex. The subsequent changes in kidney structure must depend on how much permanent damage has been caused by acute embolism of these small vessels, and upon whether the vessels remain plugged with particles or are eventually washed clear. Most of the kidneys removed from hypertensive animals exhibited some signs of permanent damage and consequent local infarction.

Later reactions: Injection of Kieselguhr, after some weeks or months, gives rise to three types of lesions.

1. *Complete infarction.*—This type of lesion was never found associated with hypertension.

2. *Partial focal necrosis.*—This occurred as a result of patchy obstruction of some medium-sized vessels as well as the arterioles and capillaries. In most organs the Kieselguhr particles were visible scattered over the cortex, usually plugging the afferent glomerular vessels and the glomerular and tubular capillaries. In some sections the smaller branches of the arcuate arteries were

DESCRIPTION OF PLATE.

FIG. 2.—Macroscopic changes (*a*) in the injected kidney after two months. (*b*) Was not injected.

FIG. 3.—Diagram of vascular arrangement in the region of the glomerular tuft. (See text.)

FIG. 4.—Effect of injection of Kieselguhr suspension. Note the clearing of the cortico-medullary boundary in the injected kidney (*a*). (Vessels injected with carmine-gelatine.)

FIG. 2.

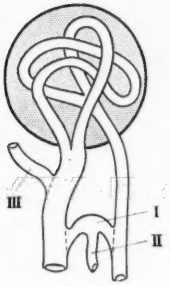
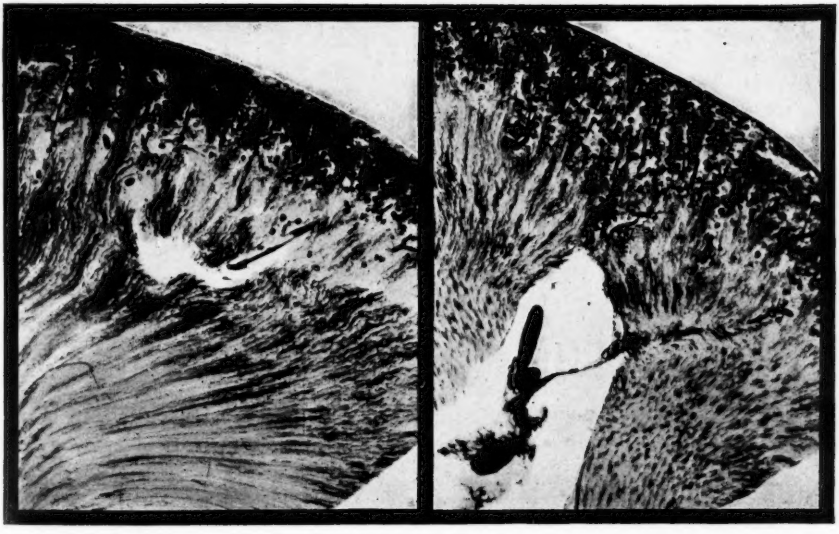


FIG. 3.



(a) FIG. 4. (b)

also occluded, producing wedge-shaped regions of necrotic or fibrotic tissue. Over the fibrotic areas the capsule was thickened and adherent. There were no marked histological changes in the vessels of either the injected or normal kidney. This type of lesion was always associated with hypertension.

3. *Obstruction of small vessels only.*—The vessels obstructed by Kieselguhr in this type of lesion included the afferent arterioles of the glomerular tufts, the capillaries of the tufts and the efferent arterioles, leading either from the glomeruli or directly from the afferent vessels. In any one kidney any or all of these vessels might be affected, but the observed lesions could be subdivided roughly into (i) those in which the cortical afferents and capillaries were chiefly involved, and (ii) those in which the efferent vessels were mainly affected. This distinction was made clearer by injection of the vessels with carmine-gelatin. Study of normal kidneys prepared by this method showed that the circulation through the glomerulus could be interfered with either by obstruction of the glomerular vessels, or by short-circuiting the blood-flow. Obstruction may occur in the afferent glomerular arteriole, the glomerular tuft, or the efferent arteriole. Short-circuiting of the blood-flow can occur as the result either of obstruction or some alteration in the calibre of the vessels, resulting from thickening of the walls of the arteries or arterioles, or from vascular spasm. The channels through which the short-circuited blood can pass are shown diagrammatically in Fig. 3 and may be listed as follows : (i) Arterioles linking the afferent arteriole with the glomerular efferents or other arterioles. (ii) Arterioles arising from the small branches of the interlobular vessels and from the afferents themselves, and passing directly into the medulla parallel to the tubules. (iii) Arterioles arising from the afferent arterioles and passing outwards to the subcapsular network of vessels. Injected Kieselguhr involved all these vessels in some of the kidneys examined.

An example of the type of lesion arising after injection from the blockage of the efferent vessels passing from the cortex to the medulla is to be seen in Fig. 4. The animal concerned developed hypertension. Note that the small vessels of the affected kidney are well injected with the carmine gelatin except in the corticomedullary region, where many of the glomerular tufts are badly injected or not injected at all. These glomeruli are more cellular than normal and are often shrunken with dilated capsular spaces. No Kieselguhr was discovered in this specimen. Van Giesen staining reveals a patchy fibrosis in the cortico-medullary region, where there are many fewer vessels than in the normal kidney. Some of the vessels present in this region exhibit endothelial proliferation. The tubules of the cortex show little change, but in the medulla there is some degree of atrophy, with interstitial fibrosis extending down from the cortico-medullary region. The larger vessels of the kidney show no changes.

General summary of histological findings.

The degree of change resulting from Kieselguhr injection into the renal artery thus varied from complete necrosis of the whole organ (not associated with high blood pressure) to interference with the circulation through the cortical and corticomedullary arterioles and capillaries, followed by slight patchy fibrosis of the associated kidney substance. The changes in the

glomeruli and tubules appeared to be secondary to the vascular changes in most kidneys examined, although it has not been possible to exclude any direct damaging action of the Kieselguhr. The degree of damage to the renal tissue was not, however, the deciding factor in the development of hypertension, as in some animals in which the blood pressure rose considerably there was very little alteration of the parenchyma. (See, e.g., Fig. 4.) On the other hand, obstruction of the kidney vessels seemed to be constantly associated with the development of hypertension, although complete obstruction of vessels leading to massive infarction of the organ was not.

II. THE CONTROL OF KIESELGUHR HYPERTENSION.

Various operative procedures were found to be successful in reducing the hypertension produced by intra-renal injection of Kieselguhr White.

1. *Nephrectomy.*

Excision of the affected kidney in two animals resulted in a fall of blood pressure to normal levels. This reduction in blood pressure was not immediate, beginning one to two days after nephrectomy and becoming complete in 6 to 8 days. (See Fig. 5.)

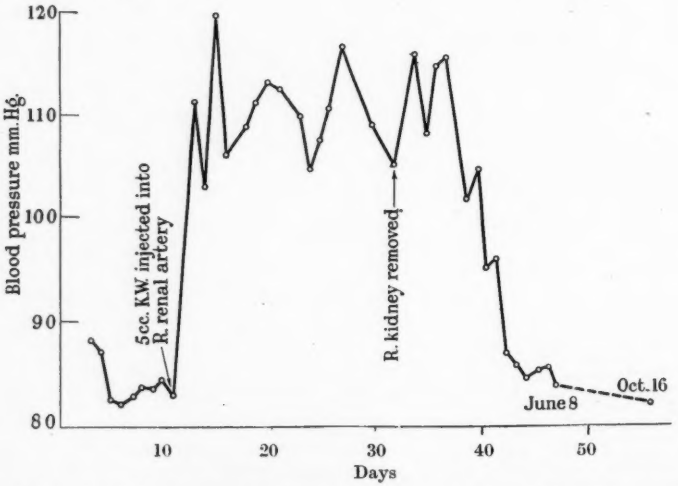


FIG. 5.—Effect of unilateral nephrectomy on hypertension subsequent to unilateral intrarenal injection of Kieselguhr suspension.

The disappearance of hypertension after removal of the affected kidney indicated that the hypertension produced by the Kieselguhr injection was of renal origin. The fall of blood-pressure was not due to the reduction in total renal substance, for unilateral nephrectomy in two normal animals resulted in only slight and inconstant lowering of the blood-pressure level. This slight

fall of blood pressure after nephrectomy in normal animals has also been noted by Gravitz and Israel (1879).

2. *Denervation following Kieselguhr Injection.*

(i) *Sympathectomy plus renal and adrenal denervation.*

Two animals which had been rendered hypertensive by unilateral Kieselguhr injection had the following operative procedures performed on them at a single operation and on the injected side only : (*a*) The abdominal sympathetic chain was removed from the crust of the diagram to the pelvic brim, and the connections of the ganglia were pulled out as far as was conveniently possible. (*b*) The splanchnic fibres running to the adrenal were excised. (*c*) The kidneys were denervated by excision of the nerve tissue of the renal pedicle, by stripping the adventitia from the renal artery until the vessel presented a long fusiform aneurysm, or a series of aneurysms, and finally by interrupting all possible connections of the kidney except those along the ureter, which was not cleaned. Following these operations the blood pressure fell to normal in each animal in 3 to 5 days.

(ii) *Sympathectomy plus renal denervation only.*

One animal with a moderate degree of hypertension which had persisted for 139 days, following unilateral injection of Kieselguhr, had the abdominal sympathetic chain removed and the renal pedicle stripped on the injected side. For 20 days after the operation the pressure fluctuated between normal and hypertensive levels. Laparotomy revealed a bundle of intact nervous tissue on the posterior surface of the pedicle. This was cut and the blood pressure fell slowly to normal in 8 days and remained normal until the animal was killed 74 days later. It is to be noted that in this case the nervous supply to the suprarenal gland was not directly disturbed.

(iii) *Renal denervation.*

Seven animals which had developed hypertension following unilateral Kieselguhr injection had the pedicle of the affected kidney stripped. In six of these animals the blood pressure returned to normal, the reduction of pressure beginning in 1 to 5 days and becoming complete in 4 to 8 days. (See Fig. 6.) The seventh animal maintained a steady hypertensive blood pressure following the supposed denervation, but after 16 days further operation disclosed a large bundle of intact nervous tissue on the posterior aspect of the artery. Section of this bundle resulted in a fall of blood pressure to normal levels in 3 days. (See Fig. 7.)

The blood pressures of all these seven animals remained within normal limits until they were killed or some other operative manoeuvres were undertaken.

An eighth rabbit, with a hypertension which had lasted 230 days after bilateral Kieselguhr injection, had both renal pedicles stripped at one operation. The blood pressure remained elevated for 4 days following this operation, but had fallen to normal by the 7th day after denervation.

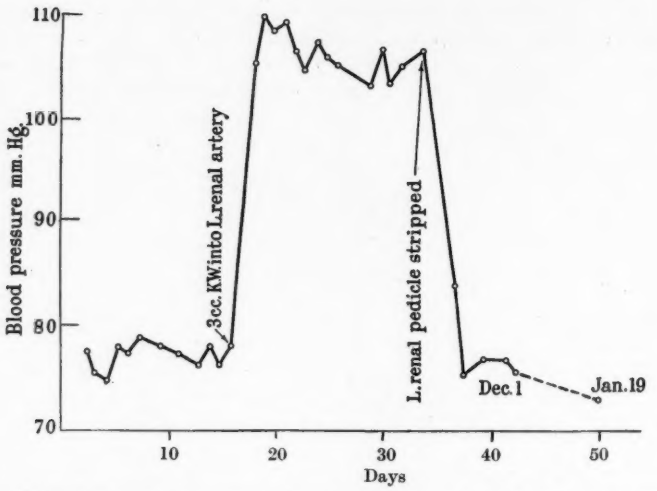


FIG. 6.—Effect of stripping the nervous tissue from the pedicle of the Kieselguhr-injected kidney. Note the fall in blood pressure to normal limits over a period of days.

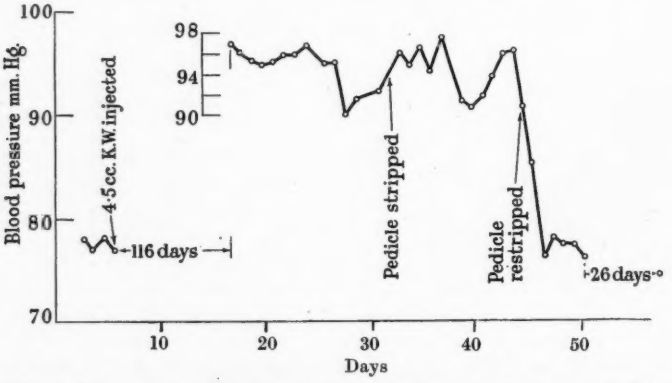


FIG. 7.—Effect of incomplete stripping of nervous tissue from renal pedicle. In this case a bundle of nervous tissue was accidentally left *in situ* at the first operation. Subsequent removal of this bundle resulted in an immediate fall of blood pressure to normal limits.

3. *Denervation preceding Kieselguhr injection.*

(i) *Renal denervation immediately preceding injection.*

The left renal pedicle was stripped of nervous tissue in five animals and an injection of Kieselguhr White suspension was made into the renal artery immediately afterwards. A prompt elevation of blood pressure occurred in all these animals, reaching an extent of 25 to 40 mm. Hg in 1 to 3 days and

returning to normal levels by the 4th to 8th day. With the exception of one animal which showed a subsequent smaller rise from the 9th to 13th day, all the blood pressures of these rabbits remained normal until death or further operation 51 to 150 days later. A typical pressure response is shown in Fig. 8.

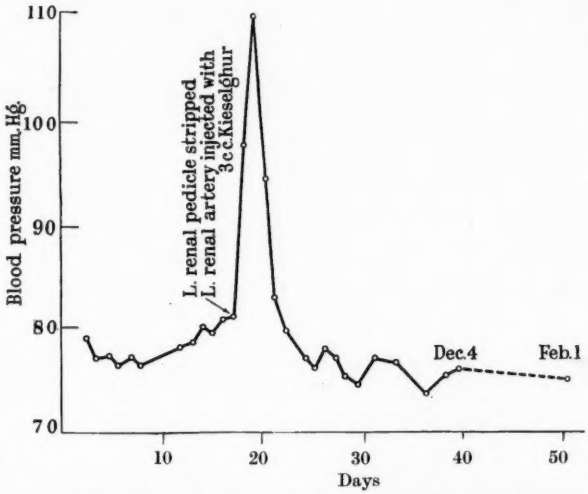


FIG. 8.—The renal pedicle was stripped of nervous tissue before the Kieselguhr suspension was injected into the artery. Note that only the spike rise of blood pressure has developed.

We considered this spike-like elevation in the blood pressure following injection of Kieselguhr into the denervated kidney to be the same phenomenon as the sharp initial rise observed in most animals after injection of the intact kidney. In the denervated animals the subsequent permanent rise of blood pressure fails to develop, suggesting that this second, more slowly developing hypertension may be dependent for its existence upon an intact nervous mechanism. It must be noted in support of this that the histological picture exhibited by four out of five animals discussed in this section closely resembled that found in the kidneys of animals rendered permanently hypertensive by injection of Kieselguhr into the innervated kidney. The left renal artery of the fifth animal was later constricted and the kidney subsequently underwent atrophy fibrosis. The kidney of the fifth animal exhibited no histological change from normal.

(ii) *Bilateral sympathectomy with renal and adrenal denervation.*

The abdominal sympathetic chains of two rabbits were removed, the adrenals denervated and both renal pedicles stripped at the one operation. Three weeks after this operation carotid loops were made and the blood pressures subsequently recorded. Five months later the left renal arteries

were injected with Kieselguhr White suspension. The resulting blood-pressure responses were irregular. The initial spike-like rise and fall of blood pressure occurred in each case, and was immediately succeeded by a rise to a level of over 40 mm. Hg above normal. In both animals, however, when the hypertension appeared to be well established (three weeks after Kieselguhr injection) the pressure suddenly fell to normal level, where it remained until the animals were killed three and five months later respectively. The explanation of this peculiar response is obscure.

DISCUSSION.

Unilateral or bilateral intrarenal injection of a suspension of Kieselguhr White results in a sharp rise and fall of blood pressure ("spike"), succeeded by a slower rise to a permanent high level. The "spike" phase was obtained by intrarenal injection of normal saline as well as Kieselguhr, and was observed in almost every animal in which the blood supply of the kidney was interrupted by any intrarenal injection. It thus appears to be associated with circulatory interruption and is probably caused by the temporary renal ischaemia, and therefore similar to the transient elevation of blood pressure obtained in dogs by Verney and Vogt (1938) by brief intermittent compression of the renal artery.

The prolonged nature of the transitory rise in the two animals which were denervated several months before the injection of Kieselguhr might thus have been caused by permanent changes in the circulation resulting from denervation (Milles, Muller and Petersen, 1932), such prolonged changes having brought about an altered sensitivity either to the temporary interruption of the blood-flow or to the immediate effects of the injected Kieselguhr. A somewhat similar response was given by Rabbit 2289, which had the kidney denervated and Kieselguhr injected at the same time.

The permanent hypertensive phase does not appear to be due directly to reduction of renal substance or to a permanent detectable disturbance of renal function, although there is normally an associated reduction in the size of the injected kidney. The hypertension is, however, dependent for its persistence upon an intact nervous supply to the kidney, even though it is slow in onset and in termination after section of the renal nerves. No rise of blood pressure occurs if the injected kidney has been completely infarcted, hypertension appearing only if there is a certain amount of renal tissue remaining. The delay in onset of this hypertensive phase of hypertension, and the slow return of blood pressure to normal* after section of the renal nerves, suggests that the rise of blood pressure is not brought about by a simple reflex mechanism acting on the systemic blood pressure as a result of afferent impulses coming from the kidney. This is further borne out by acute experiments on both rabbits and cats in which we failed to demonstrate any greater increase of blood pressure following electrical stimulation of the renal nerve trunks than could be obtained purely by mechanically clamping the renal or mesenteric arteries. Thus, although a general reflex mechanism has not been absolutely excluded, it

* This slow return to normal limits may be partly explained by interference with the kidney circulation during the denervating operation.

seems probable that the injection brings into play some local reflex in the kidney substance itself, acting on the small vessels and reducing the blood-flow through them, thus giving rise in the undamaged renal tissue to a condition of local ischaemia similar to that induced by constriction of the renal artery (Goldblatt *et al.*, 1934). The presence of some such local reflex mechanism is suggested by the work of Milles, Muller and Petersen (1931, 1932), who demonstrated vasoconstriction in the kidney as a result of the administration of snake venom, such vasoconstriction being absent in denervated kidneys. The stimulus for this reflex is probably supplied by the damage caused to small vessels by the passage through them of the Kieselguhr particles or by their actual blocking by the Kieselguhr. A constant supply of afferent impulses is set up in the nerve endings in the fibrosed vessels, bringing about constriction of the minute vessels in the undamaged renal tissue and thus creating the state of local ischaemia. Section of the nerve trunks running along the renal arteries breaks the reflex path, allowing the constricted vessels to return to their normal bore and ultimately restoring the blood-flow to the affected part, causing the hypertension to disappear.

The failure of the hypertension to develop after complete infarction of the kidney following injection of Kieselguhr further supports this view, since in this condition no normal renal tissue exists in the damaged organ and relative ischaemia cannot be produced. Further, this absence of hypertension in infarcted kidney experiments demonstrates that it is not the mere presence of Kieselguhr which initiates the hypertension.

Thus it appears that the hypertension produced by intrarenal injection of Kieselguhr suspension is initiated by a mechanism essentially similar to that which obtains in Goldblatt's ischaemic kidney experiments.

SUMMARY.

1. High blood pressure can be produced in the rabbit by unilateral intrarenal injection of Kieselguhr White.

2. Some kidney tissue must be present after injection before hypertension is developed. Complete infarction of the kidney by Kieselguhr is not followed by a rise in blood pressure.

3. The hypertension induced by injection of Kieselguhr exhibits two phases, viz. (*a*) a sharp rise—spike—immediately after injection, and (*b*) a slower permanent rise.

4. The spike rise appears to be due to temporary ischaemia produced by traction on the renal artery at the time of injection.

5. The spike rise is unaffected by section of the renal nerves either before or after injection of the Kieselguhr, but the slower developing second hypertensive phase is terminated by section of the renal nerves and does not appear if the nerves are cut prior to injection.

6. The second phase of hypertension is probably caused by local reflex constriction of the kidney vessels which gives rise to localized partial ischaemia.

7. Injection of Kieselguhr into the splenic artery has no effect upon the blood pressure.

REFERENCES.

APFELBACH, C. W., AND JENSEN, C. R.—(1931) *J. clin. Invest.*, **10**, 162.
CASH, J. R.—(1924) *Johns Hopkins Hosp. Bull.*, **35**, 168.
CONWAY, E. J.—(1933) *Biochem. J.*, **27**, 430.—(1934) *Ibid.*, **28**, 283.—(1935*a*) *Ibid.*, **29**, 2755.—(1935*b*) *Ibid.*, **29**, 222.
Idem AND BYRNE, A.—(1933) *Biochem. J.*, **27**, 419.
FERRIS, H. W., AND HYNES, J. F.—(1931) *J. lab. clin. Med.*, **16**, 597.
FRIEDMAN, L., AND WACHSMUTH, W.—(1930) *Arch. exp. Path. Pharmak.*, **150**, 173.
GOLDBLATT, H., *et al.*—(1934) *J. exp. Med.*, **59**, 347.
GRAWITZ, P., AND ISRAEL, O.—(1897) *Virchows Arch.*, **77**, 315.
JANEWAY, T. C.—(1909) *Proc. Soc. exp. Biol.*, **6**, 109.
KATZENSTEIN, M.—(1905) *Virchows Arch.*, **182**, 327.
MACLEAN, F. J., AND MAEGRAITH, B. G.—(1939) *J. Physiol.*, **95**, 7P.
MAEGRAITH, B. G., AND CARLETON, H. M.—(1939) *J. Path. Bact.*, **48**, 33.
MAEGRAITH, B. G., AND MACLEAN, F. J.—(1938) *J. Physiol.*, **92**, 44P.
MARK, R. E.—(1928) *Z. ges. exp. Med.*, **59**, 601.
Idem AND GIESENDORFER, H.—(1930) *Ibid.*, **74**, 350.
MILLES, G., MULLER, E. F., AND PETERSEN, W. F.—(1932) *Arch. Path.*, **13**, 233.—(1931) *Proc. Soc. exp. Biol.*, **28**, 561.
PICKERING, G. W., AND PRINZMETAL, M.—(1938) *Clin. Sci.*, **3**, 357.
PILCHER, J. D.—(1913) *J. biol. Chem.*, **14**, 389.
SCARFF, R. W., AND MCGEORGE, M.—(1937) *Brit. J. exp. Path.*, **18**, 59.
SENATOR, H.—(1911) *Z. klin. Med.*, **72**, 189.
VAN LEERSUM, E. C.—(1911) *Arch. ges. Physiol.*, **142**, 377.
VERNEY, E. B., AND VOGT, M.—(1938) *Quart. J. exp. Physiol.*, **28**, 253.
WOOD, J. E., AND CASH, J. R.—(1936) *J. clin. Invest.*, **15**, 543.

ELECTRICAL CHANGES IN WOUNDS AND INFLAMED TISSUES. PART I: THE BIOELECTRIC POTENTIALS OF CUTANEOUS WOUNDS IN RATS.

H. BURROWS, J. IBALL AND E. M. F. ROE.

From the Chester Beatty Research Institute, The Royal Cancer Hospital (Free), London, S.W. 3.

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THE work of various investigators on the mechanical phenomena of inflammation was summarized a few years ago by one of us (Burrows, 1932). Although the investigations then referred to and others of the same kind have made familiar the more obvious results of inflammation, the forces on which these depend have not yet been demonstrated finally. Exudation, oedema, stasis and diapedesis are among the events which seem to demand physical interpretation, and several facts suggest that an important dynamic agent in these phenomena is electrical.

It is generally recognized that differences of potential are intimately concerned with vital activities. Nearly a hundred years ago du Bois-Reymond (1848) showed that in injured muscle and nerve a potential difference exists between the injured and uninjured tissues, such that the injured part is positive with respect to the uninjured part when the direction of current within the muscle is considered, or negative when measured in the external galvanometer circuit. Since then the electrical changes which accompany the activity of muscle and nerve have been studied in very great detail.

There seems to have been a tendency to assume that trauma of other tissues would cause local changes of potential of the same character as those which occur in muscle and nerve. In any event, it is not easy to describe the potentials in a living tissue in terms which are simple and at the same time quite free from ambiguity. Other observers, e.g. Lund (1928) and Hyman and Bellamy (1922), have called attention to consequent lack of uniformity in recording experimental results. The potential difference between wound and normal tissue may be described either in terms of the direction of the current within the body, or its direction through the galvanometer. These two methods of description would lead to opposite conclusions as to the signs of the potentials, and it is easy to see how misapprehension of results might occur. Throughout the following paper the potential differences will be described as indicated in the galvanometer circuit, and it will be seen that the negative potential which occurs in nerve and muscle in response to injury is not a general phenomenon in every living tissue.

Long ago it was recognized that the deep surface of the frog's skin is

positive to the outer surface (Bernstein, 1912), and hypothetical explanations of this fact seem to have been accepted without critical examination of their force or experimental proof of their soundness. More recently Burr, Harvey and Taffel (1938) found in guinea-pigs that a wound of the skin showed a positive potential as indicated in the galvanometer circuit, compared with the uninjured surface of the skin nearby, and Burr, Smith and Strong (1938) say that cuts and abrasions of the skin in humans also produce marked voltage rises, the cut area being " as often positive as negative " to the normal skin surface. (See also Burr, Taffel and Harvey, 1940.)

So far as concerns the transport of matter through the walls of the blood vessels in regions of inflammation, it is known that the leucocytes, proteins and most of the other suspended or colloidal components of the blood are negatively charged in that medium. Abramson, who has reviewed the work of earlier observers (1927, 1928, 1934), and has himself investigated some of the physical aspects of the problem (1924, 1925), concluded that " the high gradient of potential existing between injured and uninjured tissues could conceivably account partially for the migration of charged polymorphonuclear leucocytes in a directed fashion towards a point of injury."

The disposal of foreign particles after their injection into the blood-stream is another event which seems to demonstrate that inflammation is accompanied by local electrical changes, for the distribution of such particles in the body largely depends upon the electrical charges which they carry. In normal conditions fine suspended particles or colloidal substances carrying a negative electric charge in a watery medium when injected into a vein circulate freely until they are arrested and removed from the blood by the reticulo-endothelial organs. If a focus of inflammation is present the substances mentioned adhere to the walls of the small blood vessels in the region of inflammation, and later traverse them so as to become concentrated in the tissue and tissue spaces outside the vessels ; here the extravasated materials are retained, whereas if introduced, e.g. by injection, in the absence of inflammation they drain away.

Various tests with dyes have shown that only those substances which carry an electro-negative charge become abstracted in this way from the blood by the reticulo-endothelial organs and inflamed subcutaneous tissues. An electro-positive dye which is not rapidly reduced in the blood, e.g. night blue, when introduced into the blood-stream does not circulate freely. It adheres to the normal vascular endothelium, and shows relatively little tendency to become arrested by the reticulo-endothelial organs or by the blood vessels of inflamed tissue (unpublished results). Moreover electropositive colloids when injected beneath the skin themselves act as inflammatory agents.

A consideration of all the facts mentioned above suggested the desirability of determining the electrical changes which accompany wounds and inflammation, and of correlating them, if possible, with the associated pathological changes.

The first step in this inquiry has been to determine the normal variations which may be observed in closely related sites on the normal uninjured skin, and later to note the differences of potential which are shown when a wound is inflicted at one of the chosen sites. In the first instance such observations were made on rats.

TECHNIQUE.

The apparatus employed in these experiments is a vacuum tube microvoltmeter, similar to that described by Burr, Lane and Nims (1936) for the measurement of steady state potential differences in living organisms. The instrument has a high sensitivity, sufficiently high input impedance, and is

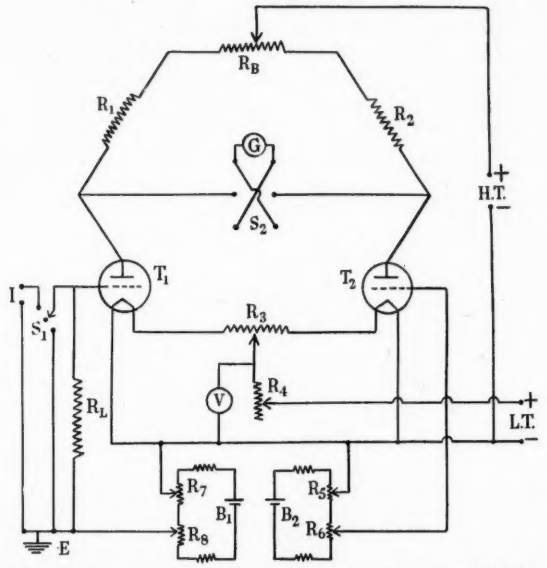

FIG. 1.—Circuit diagram of vacuum tube microvoltmeter. $R_1 = R_2 = 10{,}000$ ohms; $R_3 = 2500$ ohms; $R_3 = 1$ ohm; $R_4 = 2$ ohms; $R_5 = R_7 = 200$ ohms; $R_6 = R_8 = 20{,}000$ ohms; T_1 and T_2 = Cossor radio valves (220 P); $B_1 = B_2 = 1{\cdot}5$ volts; L.T. = 2 volts; H.T. = 40 volts; I = Input terminals; $R_L = 10$ megohms.

but little affected by external electrical disturbances. The current drawn from the animal under measurement is reduced to a minimum, and the only shielding required is provided by placing all the apparatus on an earthed copper sheet.

The potential difference to be measured is applied to the grid of the valve T_1 (Fig. 1), to which a pair of non-polarizable electrodes is attached through the input terminals at I. Pipettes filled with saline connect the measuring-points on the animal with the electrode-system. Before measurements are made, the valves T_1 and T_2, which are arranged in two arms of a Wheatstone network, are balanced by varying the grid voltages B_1 and B_2, so that when no potential difference is applied to T_1, no current flows through the galvanometer G. When a potential is applied to the grid of T_1, the galvanometer shows a deflection which can be calibrated by means of a potentiometer and standard cell.

Apparatus.

A critical study of the type of circuit used in this work has been given in a paper by Nottingham (1930), and this was consulted in designing the present instrument, which differs in certain respects from that of Burr. The circuit-diagram is shown in Fig. 1. Cossor radio valves type 220 P were used, and these were obtained in pairs which had been roughly matched for similarity in anode impedance. The filaments were run at approximately 1·7 volts and one pair of valves has been in constant use for over two years. As recommended by Nims (1938), a separate variable grid voltage has been supplied for the balance valve T_2, thus making the circuit more nearly symmetrical and ensuring greater stability.

In order to reduce the drain on the grid bias batteries a balance was first obtained by using only the two variable resistors R_7 and R_8. Suitable fixed resistors of about 10,000 ohms each were then inserted on either side of the variable resistors, so that the latter had a sufficient range about their mean positions. The grid bias batteries were left permanently connected in circuit, $1\frac{1}{2}$ volts across approximately 40,000 ohms. One large capacity torch battery has been left in each grid bias circuit for over two years and still seems to be efficient.

All the variable resistors were made by General Radio. The fixed resistors are precision wire-wound, and the 10 megohms grid leak is a first quality Siemens-Schuckert type. The inclusion of the variable resistance R_B makes it possible to adjust the circuit so that the balance is sensibly independent of reasonable changes in the H.T. battery voltage (Nottingham, 1930). No switches have been used in the battery circuits, since they are liable to be a source of trouble, especially in the L.T. circuit. The switch S_1 is a General Radio product and was found to possess excellent insulation, especially when the bakelite is coated with a thin layer of wax. Amber insulation, as advocated by Burr *et al.*, was found to be unnecessary. The reversing switch S_2 was incorporated to enable a more open scale to be used while retaining a compact galvanometer.

For the experiments described in this paper, sensitivities of 70 microvolts per millimetre deflection or a tenth or a hundredth of this value have been found convenient, and were obtained by the use of a Tinsley galvanometer (resistance 1200 ohms, sensitivity 300 mms./microamp.) and universal shunt. The instrument was calibrated for each range of the shunt by applying to the input terminals known voltages from a potentiometer. This was done at frequent intervals, but after settling down the changes from time to time in the calibration were almost negligible. The electrode system consisted of two silver-silver chloride electrodes immersed in 0·9 per cent. sodium chloride solution. One electrode was earthed, and the signs of the potentials at the wounding-point were therefore positive or negative with respect to this reference electrode.

Experimental procedure.

Wistar rats of varying ages were used for these experiments, i.e. 10 rats weighing 25–30 g., seven rats weighing 200–350 g., and 41 rats weighing 100–150 g. each ; seven were females and 51 were males. Each animal was anaes-

thetized by means of an intraperitoneal injection of avertin, in order to immobilize it during the measurements. It was then placed on a cork board, which was on an earthed plate ; the hair was clipped to the skin over the anterior ends of the ilia and the midline of the back, the skin moistened with saline at the measuring positions, and the electrode pipettes were lowered into the salt pools. Three pairs of measuring points were used in each animal ; i.e. from a point D (Fig. 2) where the wound was to be made on the midline of the back, midway between the interscapular cleft and the anterior ends of the ilia, to reference points A and B on the skin over the anterior ends of the ilia, and C on the midline of the back, about 1 cm. behind D. The potential differences DA and DB were measured in 58 animals and DC in 36 of them.

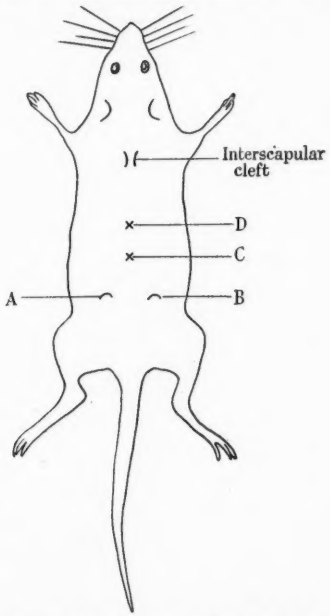

FIG. 2.—Diagram of rat showing points between which potential differences were measured.

In order to determine the normal change of potential over a small period of time, measurements were made over a 2-minute and a 5-minute interval, before wounding the animals at D. Wounding and the subsequent measurement of the potential differences occupied from 1 to 5 minutes, and the potential changes over 2-minute and 5-minute intervals were then remeasured. Similar measurements were repeated on the day following wounding.

The wounds were initially uniform throughout the series of rats. In each case a circular disc of skin, 6 to 7 mm. in diameter, and including the underlying panniculus carnosus, was removed by means of scissors. The wounds were unsutured, and received no applications other than the saline used when the measurements of potential were being made.

RESULTS.

The potential differences DB, DA and DC immediately before and after wounding are shown for all the animals investigated in Figs. 3 to 5. Ordinates represent potentials in millivolts, and the vertical lines drawn in the figure therefore show the changes of potential which occur in the different animals on wounding. The potential changes in all the animals for the pair of measuring points DB are shown in Fig. 3, and are arranged from left to right in decreasing

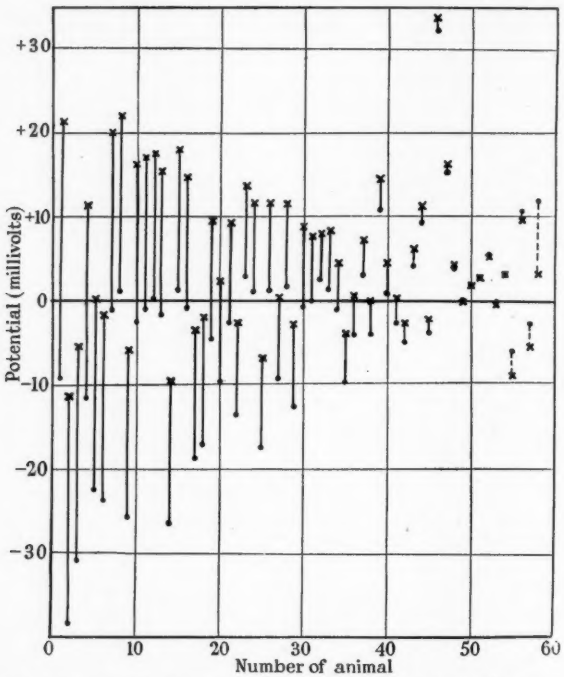

Fig. 3.—Potential difference DB, between wound-point D and reference-point B, immediately before and after wounding. ● = p.d. before wounding. × = p.d. after wounding.

order of magnitude of positive potential-change. Each animal therefore is designated by a number, 1 to 58, in Fig. 3, and the same individuals are placed in the same order from left to right in the subsequent Figs. 4, 5 and 6.

It is seen from Fig. 3 that the amount of potential change on wounding, for the position DB, varied considerably for different animals (from −9 to +30 mv.). It seems clear, however, that in general the potential of D, with respect to the uninjured spot, B, which was earthed, became positive on wounding. In four animals (Nos. 55–58) a change in a negative direction occurred, and in six animals (Nos. 49–54) no potential change occurred on wounding.

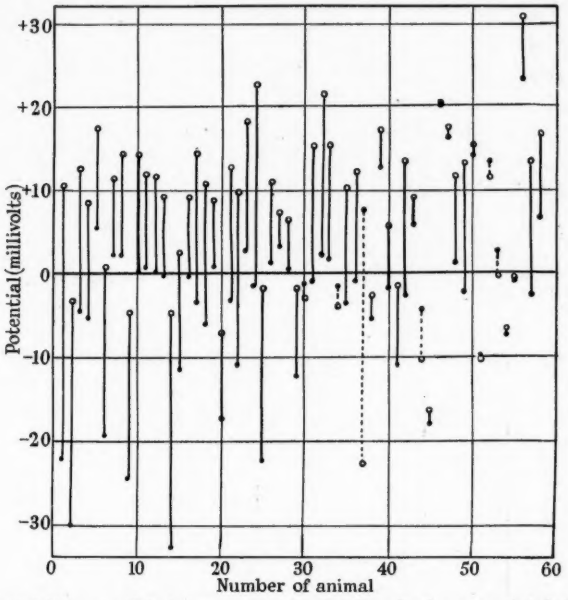

FIG. 4.—Potential difference DA, between wound-point D and reference-point A, immediately before and after wounding. ● = p.d. before wounding; ○ = p.d. after wounding.

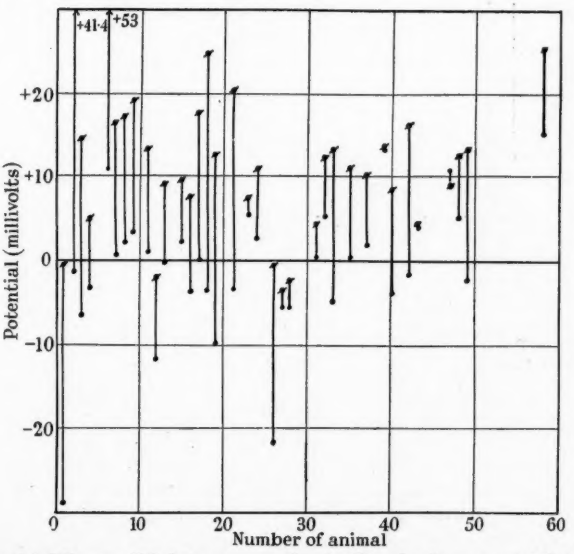

FIG. 5.—Potential difference DC, between wound-point D and reference-point C, immediately before and after wounding. ● p.d. before wounding; ◉ p.d. after wounding.

From Fig. 4 in which the potential difference DA, before and after wounding, is shown for all the animals, it is apparent that there was a tendency for this potential to follow the same course as DB, on the opposite side of the body, though there were obvious deviations from this in some animals. On wounding, the potential of D with respect to A became more negative in six of the animals (Nos. 30, 34, 37, 44, 52 and 53)—all different from those giving a negative change at DB—and showed no change in two animals (Nos. 46 and 55).

Fig. 5 shows the potential differences DC for the 36 animals measured; there was again considerable variation (from 0 to 42 mv.) in the magnitude of the positive potential on wounding. In one animal (No. 47) D became negative with respect to C, and in two animals (Nos. 39 and 43) there was almost no potential change on wounding.

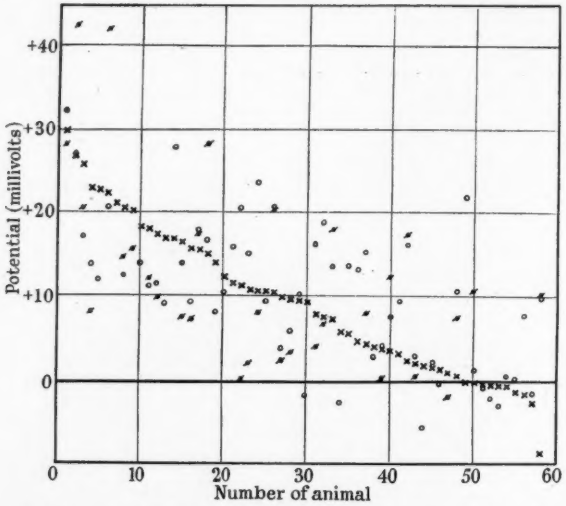

FIG. 6.—Changes in potential differences DB, DA and DC on wounding. × change in p.d. D.B.; ○ change in p.d. DA; ● change in p.d. DC.

There is a tendency for the value of the initial potential-difference to affect the magnitude of the change in potential which occurs on wounding. This is evident from the results shown in Figs. 3 and 4, where it is seen that the largest potential-changes on wounding (plotted at the left-hand side of the figures) are shown by those animals having a large negative potential initially at DB and DA.

The actual potential changes at DA, DB and DC, on wounding, are shown in Fig. 6. They can be compared with the changes which occurred in a 5-minute interval before wounding, Fig. 7, and in a similar period after wounding, Fig. 8, arranged, in both graphs, from left to right in decreasing order of magnitude of positive potential-change at DB. (The effect of various anaesthetics on the normal bioelectric potentials of rats has been studied by Burr and Smith (1938), who found no evidence of any profound effect by the

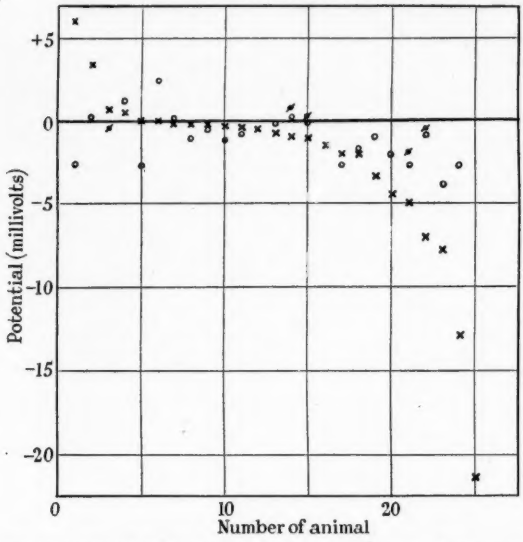

FIG. 7.—Changes in potential differences DB, DA and DC over a 5-minute interval before wounding. X change in p.d. DB; ○ change in p.d. DA; ● change in p.d. DC.

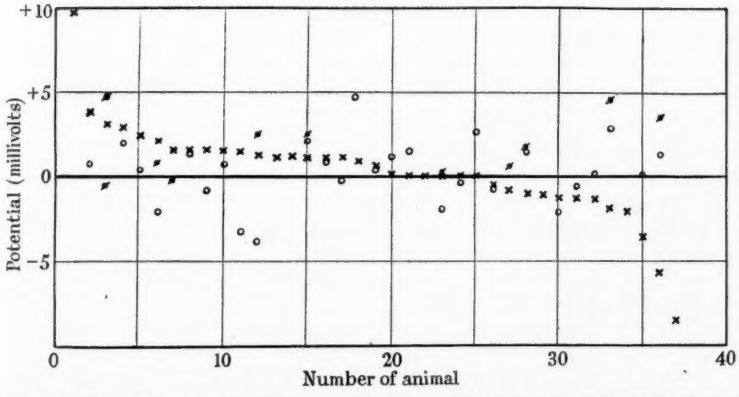

FIG. 8.—Changes in potential differences DB, DA and DC over a 5-minute interval after wounding. X change in p.d. DB; ○ change in p.d. DA; ● change in p.d. DC.

drugs.) It is seen from Figs. 7 and 8 that whereas, before wounding, the potentials tended to drift with time towards the negative, after wounding they drifted towards the positive, but in most cases these changes were small compared with the immediate potential change on wounding.

In Table I is shown, for each of the three pairs of measuring points, the *average* change of potential on wounding, together with the average changes over 2-minute and 5-minute intervals before and after wounding, for the 58 rats examined. It is clear from these figures that the average change in potential on wounding was well outside the normal drift of potential.

TABLE I.—*Potential Changes between a Wound-point D, and Earthed Reference-points A, B and C (on the back of the Rat).*

For relevant data see figure.		Average potential changes (mv.). DB.	DA.	DC.
..	Over a 2-minute interval before wounding	−2·2 (26)*	−0·36 (27)*	+0·75 (8)*
7	Over a 5-minute interval before wounding	−2·4 (25)	−0·72 (27)	+0·12 (11)
3, 4, 5, 6	Immediately on wounding (occupying 1–5 minutes)	+9·5 (58)	+10·58 (58)	+12·67 (32)
..	Over a 2-minute interval immediately after wounding	+0·24 (31)	+0·58 (34)	+0·72 (16)
8	Over a 5-minute interval immediately after wounding	+0·36 (37)	+0·78 (37)	+1·42 (21)
..	Over a 2-minute interval on day following wounding	+0·46 (20)	+0·31 (16)	+1·05 (11)
..	Over a 5-minute interval on day following wounding	+0·78 (22)	+1·18 (18)	−3·35 (4)

* Number of animals measured.

DISCUSSION.

On summarizing the experiments described above, it was evident that a result had been obtained quite different from that of the work of the earlier investigators on muscle and nerve injuries. When the direction of current in the external circuit was considered, our results showed that an injured area of the skin was positive to the uninjured skin, whereas in the earlier investigations on muscle and nerve, the site of injury was negative to the uninjured tissue. In order, therefore, to compare the signs of the potentials recorded in this paper with the results obtained by earlier workers, experiments on injured muscle have also been carried out, using the technique described above. The extensor muscles of the left and right legs and the left and right erector spinae muscles in the Wistar rat were used, the animal having been heavily anaesthetized and the skin cut and turned back over these muscles. Potential differences of −2·0 to +2·5 mv. were observed between points 1·5 to 3 cm. apart on the uninjured surface of the muscles, and on causing an injury at one of the measuring points potentials of −5 to −34 mv. developed, the injured

region being negative to the uninjured surface. These potentials were of the same order of magnitude as those observed on injuring the skin, although the sign of the potential was reversed. Measurements on the gastrocnemius muscle of the frog gave similar results, in accordance with the work of earlier investigators. (The fact that the surface of an intact muscle is positive as measured in an external circuit to the surface of its tendon has been borne in mind.)

The potential of the under surface of the skin of the frog and rat, when turned back to expose the muscle, was positive with respect to the outer surface, as has been observed by other investigators. This positive potential may, of course, be produced by injury, i.e., by turning back or cutting out a portion of the skin, or it may be that exposing the under surface of the skin reveals a potential difference normally existent between the under and outer skin surfaces.

It is clear from these supplementary experiments that there is a real difference in sign between the potential observed at an injured muscle surface and that measured in injured skin. This might explain the occasional cases recorded in this paper in which a negative potential-change was observed on injuring the skin. The skin wounds involved muscle fibres of the panniculus carnosus, which is well developed in rats. If the negative potential due to injury of these muscle fibres, or caused by chance injury of the deep muscle layer, exceeded the positive potential developed on injuring the skin, a negative potential change might be expected.

Further observations are being made on wounds in man and they accord with those in rats. It seems that the range of the potential difference immediately detected between a wound and a healthy part of the skin is independent of the extent of the wound provided that the stratum granulosum has been penetrated. A deep burn caused no greater rise of potential than a small incision. These and other matters, including the fact that mucous surfaces seem usually to be negative to adjacent skin, will be discussed more fully in a second paper.

SUMMARY.

The immediate changes in potential which occur on injuring the skin have been examined in a series of Wistar rats, taking potential differences between a superficial cut and three earthed reference-points on the skin of the back. The changes have been compared with the normal drift of potential between these measuring-points during small intervals of time comparable with the wounding period. In nearly all cases wounding causes an increase in potential, up to 42 mv., the wound being positive with respect to the uninjured region when the direction of current in the external circuit is considered.

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authors (J. I.) acknowledges that approval for the publication of this paper has been given by the Controller General of Research and Development, Ministry of Supply.

REFERENCES.

ABRAMSON, H. A.—(1924) *Am. J. med. Sci.*, **167**, 702.—(1925) *J. exp. Med.*, **41**, 445.—(1927) *Ibid.*, **46**, 987.—(1928) 'Colloid Chemistry,' 2nd ed. J. Alexander, New York, p. 701.—(1934) 'Elektrokinetic Phenomena and their Application to Biology and Medicine,' New York.

BERNSTEIN, J.—(1912) 'Elektrobiologie.' Braunschweig.

BURR, H. S., LANE, C. T., AND NIMS, L. F.—(1936) *Yale J. Biol. Med.*, **9**, 65.

BURR, H. S., AND SMITH, P. K.—(1938) *Ibid.*, **11**, 137.

BURR, H. S., HARVEY, S. C., AND TAFFEL, M.—(1938) *Ibid.*, **11**, 103.

BURR, H. S., TAFFEL, M., AND HARVEY, S. C.—(1940) *Ibid.*, **12**, 483.

BURR, H. S., SMITH, G. M., AND STRONG, L. C.—(1938) *Amer. J. Cancer*, **32**, 240.

BURROWS, H.—(1932) 'Some Factors in the Localization of Disease in the Body.' London.

DU BOIS-REYMOND, E.—(1848) 'Untersuchungen über thierischen Elektricität.' Berlin.

HYMAN, L. H., AND BELLAMY, A. W.—(1922) *Biol. Bull.*, **43**, 313.

LUND, E. J.—(1928) *J. exp. Zoology*, **51**, 265, 327.

NIMS, L. F.—(1938) *Yale J. Biol. Med.*, **10**, 241.

NOTTINGHAM, W. B.—(1930) *J. Franklin Inst.*, **209**, 287.

CORRELATION OF *IN VITRO* AND *IN VIVO* DRUG ACTION THROUGH SPECIFIC ANTAGONISTS: SULPHANILAMIDE AND *P*-AMINOBENZOATE.

H. McILWAIN.*

From the Department of Bacterial Chemistry (Medical Research Council), The University, Sheffield.

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IF the differing activities of antibacterial agents under different conditions can be rationalized, the experimental testing of new drugs for bacteriostatic or bactericidal effects under defined conditions *in vitro* will offer some advantages over their examination in experimental animals. Comparisons of the therapeutic activities of antibacterial agents with their effects on bacteria *in vitro* have frequently been made, but apart from empirical observations the differences have not been expressed quantitatively. It was early realized that a major cause of the differences was the presence of materials in the host, which antagonized the drug. Koch (1881) attempted to cure guinea-pigs of anthrax by concentrations of mercuric salts toxic to *B. anthracis* in culture media. This was ineffective, and tissue fluids also lowered the *in vitro* activity of mercuric salts, probably by combining with them (see also Fleming, 1940; Fildes, 1940). Bechhold and Ehrlich (1906) developed agents not visibly combining with tissue fluids and active *in vitro* in concentrations corresponding to 1/100 the dose toxic to the host, but again serum so lowered their activity that they were ineffective *in vivo*. With sulphanilamide the divergence has most frequently been in the opposite sense: many observers have not found *in vitro* the therapeutically applied bacteriostasis, and this has been interpreted as due to greater quantities of antagonistic agents in some cultural media than in host tissues. It is evident, therefore, that the effect of substances which antagonize the action of a drug must be quantitatively expressed before its *in vitro* action can be used as a basis for assessing *in vivo* activity.

The most important naturally occurring substance which antagonizes the action of sulphanilamide is *p*-aminobenzoic acid. The *in vitro* interacting quantities of *p*-aminobenzoate and sulphanilamide have now been investigated, and the *p*-aminobenzoate of animal materials has been determined, in order to see whether these values are consistent with sulphanilamide bacteriostasis *in vivo* at levels used therapeutically. Such a comparison is especially necessary,

* Leverhulme Research Fellow.

as Selbie (1940) and Martin and Fisher (1942) have found that, when sulphanilamide is fed or injected together with *p*-aminobenzoate in quantities many times those sufficient to annul the action of the drug *in vitro*, *in vivo* action has nevertheless ensued. An explanation of this is offered below.

EXPERIMENTAL.

Antibacterial indices.

The ratio S/P, where S is the concentration of sulphanilamide necessary for bacteriostasis in the presence of a concentration P of *p*-aminobenzoate, was called the antibacterial index of the drug (McIlwain, 1942). Table I gives the frequency with which bacteriostasis of different degrees was found with different ratios of S/P in several experiments with three strains of *Streptococcus haemolyticus* (two of group A and one of group G). Experiments were carried out in a casein-hydrolysate medium (McIlwain, 1939) and a peptone medium (Woods, 1940) by inoculating (*a*) normal medium; (*b*) media containing sulphanilamide (5×10^{-5} to 5×10^{-3} *M*) and (*c*) media with those concentrations of sulphanilamide and also varying concentrations (5×10^{-6} to $5 \times$

TABLE I.—*Degree of Bacteriostasis Produced by Different Ratios of Sulphanilamide and* p-*Aminobenzoate Concentrations.*

Organism.	Molar ratio sulphanilamide / *p*-aminobenzoate.	Frequency of different degrees of bacteriostasis: Delay (days beyond control) in appearance of visible growth.					
		0.	<1.	1.	2.	3.	4 or more.
Streptococcus haemolyticus	300–500	10	1	0	0	0	0
	2000–2500	0	4	7	0	0	0
	4000–6000	0	0	1	2	4	4
	10,000–12,500	0	0	0	1	4	6
	20,000–25,000	0	0	0	0	2	9
Bact. coli	300–500	32	0	0	0	0	0
	2000–2500	0	15	13	3	0	0
	4000–6000	0	0	15	14	2	1
	10,000–12,500	0	0	1	14	11	6
	20,000–25,000	0	0	0	1	5	17

10^{-9} *M*) of *p*-aminobenzoate. The values for the effect of a given ratio were distributed over a certain range; the distribution was not markedly correlated with the strain of organism. Delaying of growth for three days has been taken as the criterion of bacteriostasis in the following calculations, and this gives an antistreptococcal index of sulphanilamide of 4–6000. Values for *Bact. coli* (three strains) were determined in an ammonium lactate medium (Fildes, 1938); they are comparable to those of Rose and Fox (1942), Wood (1942), and Wyss, Grunbaugh and Schmelkes (1942). The variations in these authors' figures—which are within those of Table I—are probably attributable to their differing criteria of bacteriostasis.

p-*Aminobenzoate assay.*

Clostridium acetobutylicum (Weizmann) was grown from a sand-spore preparation in 5 per cent. maize-meal and subcultured several times in medium A, which is based on those of Oxford, Lampen and Peterson (1940) and Park and Wood (1942). All cultures were incubated in 5 per cent. CO_2–H_2 at 37°. Medium A consisted of the basal medium with the sterile addition of *M* glucose (1 ml.), 5 per cent. Bacto-peptone (0·5 ml.) and water (to 10 ml.). The basal medium was prepared by dissolving $(NH_4)_2HPO_4$, 1 g.; Na_2HPO_4, 0·5 g.; KH_2PO_4, 0·5 g.; $MgSO_4$, 0·1 g.; NaCl, 0·1 g.; $FeSO_4.7H_2O$, 0·01 g., and $MnCl_2.4H_2O$, 0·01 g. in distilled water (500 ml.), adjusting the pH to 7 by NaOH, boiling for 10 min., filtering and diluting to 600 ml. It was distributed in 6 ml. quantities in test-tubes, 3–5 mg. of reduced iron (B.D.H.) added to each as a freshly prepared suspension, and autoclaved. Each assay comprised a number of tubes of the basal medium to each of which was added *M* glucose (1 ml.), biotin (0·5 ml. of a solution containing 0·01 μg./ml.), the inoculum (consisting of cells from 0·3 ml. of a 48-hour culture of the organism in medium A, collected and resuspended in water), aliquots of the solution to be assayed or of *p*-aminobenzoate, and water to 10 ml. The *p*-aminobenzoate content of the unknown solutions was judged by comparison during the following three days, of the opacities of tubes containing dilutions of them, with the tubes containing known quantities of *p*-aminobenzoate. An initial slight opacity due to the inoculum disappeared during this period if no *p*-aminobenzoate had been added.

Specificity.—*p*-Aminobenzoic acid was established as the naturally occurring compound in yeast responsible for promoting growth of *Cl. acetobutylicum* in a medium similar to that described here; the only other compounds known to produce this effect are structurally closely related to the acid (Rubbo and Gillespie, 1940, 1942). During the present work about 40 compounds of nutritional interest were tested but found inactive in this respect. A mixture of amino acids delayed autolysis of the inoculum, but did not prevent this nor promote growth. Methionine, known to antagonize sulphanilamide-inhibition of *Bact. coli*, did not promote growth of *Cl. acetobutylicum*. *p*-Aminophenylacetic acid promotes growth of *Cl. acetobutylicum* (Rubbo and Gillespie, 1942), but does not antagonize sulphanilamide. Compounds of this type were not responsible for the activity of the present materials, for the anti-sulphanilamide and growth-promoting activities of specimens examined were equivalent to about the same amounts of *p*-aminobenzoate (Table III).

Materials examined.—Values are given in Table II. Plasma was from citrated human blood, collected sterilely. A maximum limit only could be given to the content of certain specimens, of which high concentrations inhibited growth of the test organism. The necessary controls, with addition of both the unknown specimen and of *p*-aminobenzoate, were performed in each case. Corpuscles were washed with saline. Urine, human, was sterilized by filtration. The Na_2SO_4 meat extract was prepared according to Deutsch, Eggleton and Eggleton (1938).

Bound p-*aminobenzoate* (see discussion).—The following conditions were optimal for liberating the bound acid: the material (1 g.) in a plugged tube

TABLE II.—p-*Aminobenzoate Assays.*

Source.	*p*-Aminobenzoate $M \times 10^{-9}$.	Sulphanilamide calculated as necessary for 3-day bacteriostasis of *Strep. haem.* $M \times 10^{-6}$.	Excess sulphanilamide constituted by concentrations of 2 mg./100 ml.
A. Plasma 1	4	2	6-fold.
Corpuscles from 1	5	2·5	5 ,,
Plasma 2	10	5	2 ,,
,, 7	2	1	12 ,,
,, 8 and 11	5	2·5	5 ,,
Corpuscles from 8	8	4	3 ,,
Plasma 3, 4, 5, 6	<10	<5	>2 ,,
,, 10 and 12	8	4	3 ,,
B. Urine 1 and 4	20	10	1·2 ,,
2	5	2·5	5 ,,
3	10	5	2 ,,
C. Ox muscle extracts (referred to original wet weight) Na_2SO_4, 35°	1	0·5	23 ,,
90°, ½ hr.	10	5	2 ,,
boiling, ½ hr.	20	10	1·2 ,,
D. Peptone-broth 1	25	12·5	*nil*
,, 2	40	20	,,
Tryptic digest broth	10	5	2-fold
Peptone (5% solution) 1	25	12·5	*nil*
,, ,, 2	50	25	,,

with conc. HCl (0·1 ml.) was autoclaved at 120° for 20 min., sterile buffer (2·9 ml.) and *N* NaOH (1 ml.) added, and suitable dilutions assayed. Preliminary experiments with plasma gave the following *p*-aminobenzoate contents: untreated, 4×10^{-9} *M*; 1 hr. at 100° with *M*/5 HCl (*cf.* Macleod, 1940), 5×10^{-8} *M*; with *M* HCl, 10^{-6} *M*; for 20 min. at 120° with *M*/10 HCl, 5×10^{-8} *M*; with *M*/5 HCl, 10^{-6} *M*; with *M* HCl, $1{\cdot}2 \times 10^{-6}$ *M*.

Properties of the active material.—The separations of Table III show the activity to be extracted by ether from aqueous solutions of pH 4·5 (a ten-fold extraction with equal volumes of solvent), and removed from the ether by aqueous Na_2CO_3. This is consistent with the activity being due to *p*-aminobenzoate. Most (Separation B) or all (Separation C) of the " bound " *p*-aminobenzoate possessed similar acidic reactions.

DISCUSSION.

The values of Table III show that the *p*-aminobenzoate of the blood and urine specimens examined would need 10^{-5} *M* to 10^{-4} *M* sulphanilamide to prevent growth of streptococci for three days. The minimal blood concentration of the free drug normally attained in practice—2 mg./100 ml. or $1{\cdot}2 \times 10^{-4}$ M—is therefore in excess of that needed for this degree of bacteriostasis,

usually by a reasonable margin. This is presumptive evidence of the correctness of the present method of assessing drug activity in terms of antagonistic substances. The greater sulphonamide-antagonism of tissue preparations, such as bacteriological media, can, however, be interpreted also in terms of *p*-aminobenzoate, as these have been found to contain relatively large quantities of the substance in a form available to *Cl. acetobutylicum* and *Strep. haemolyticus* after digestion with acid. The reactions of this " bound " ***p*-aminobenzoate** in plasma are unexpected (*cf.* Blanchard, 1941); it also is largely an ether-soluble acid. In view of the known acetylation (Harrow, Mazur and Sherwin,

TABLE III.—*Bound* p-*Aminobenzoate Assays and Properties.*

Source.	*p*-Aminobenzoate ($M \times 10^{-9}$), Initially.	After acid treatment.
Plasma 1	4	1200, 1500*
,, 7	2	120, 200*
,, 11	5	125
Urine 2	5	500
,, 3	10	2000
,, 4	20	2000
Separation A.		
Plasma 8	5	500, 600*
Aqueous residue from ether extraction at pH 4·5 of acid-treated plasma	—	50
Ether extract of acid-treated plasma	—	300
Na_2CO_3 washings of ether extract	—	300
Separation B.		
Aqueous residue from ether extraction of untreated plasma 8 at pH 4·5	<1	<10
Ether extract at pH 4·5	3	300
Na_2CO_3 washings of ether extract	3	300
Ethereal residue from Na_2CO_3 washings	1	10
Separation C.		
Plasma 7	2	120
Ether extract of untreated plasma at pH 4·5	2	60
Aqueous residue from ether extraction	1	40

* Determined by anti-sulphanilamide activity.

1933) or other conjugation (Quick, 1932; Strauss, Lowell and Finland, 1941) of *p*-aminobenzoate on feeding or injecting to animals, it is probable that the bound *p*-aminobenzoate is *p*-acetylaminobenzoate or an analogous derivative. The acetyl compound would have the observed chemical properties and is

almost inactive as a sulphonamide antagonist (Woods, 1940). If the entire *p*-aminobenzoate of blood was uncombined, sulphanilamide in all the cases examined would be inactive.

Values for the excess of sulphanilamide constituted by therapeutic levels would necessarily be altered if normal tissues contained significant amounts of sulphonamide antagonists other than *p*-aminobenzoate. This is not considered likely, as the *p*-aminobenzoate and anti-sulphonamide activities of specimens tested have been found to tally. *p*-Aminobenzoate is *in vitro* the most active sulphonamide antagonist; methionine and purines have a lesser activity. Martin and Fischer (1942) have found adenine a more effective antagonist *in vivo* than is *p*-aminobenzoate, but this is not due to high activity on the part of adenine (of which 0·8 mg. antagonized 2 mg. of sulphanilamide) but to an unexpectedly low activity of *p*-aminobenzoate (0·8 mg. only partially antagonizing 2 mg. of sulphanilamide; 0·002 mg. are sufficient *in vitro*). A similarly low activity of orally administered *p*-aminobenzoate was reported by Selbie (1940). In neither of these investigations was the ratio between *p*-aminobenzoate and sulphanilamide the primary consideration, and, in distinction to the present experiments, the concentrations of the substances in the animals were not considered. The latent period of infection in these experiments would be expected to be sufficient for the conjugation or excretion of much of the *p*-aminobenzoate by the host (*cf.* Quick, 1932; Strauss *et al.*, 1941). It is thus an interesting possibility that, not only in these experiments, but also in normal sulphonamide therapy, the action of the drug is dependent upon the host's conjugation of much of the coincident *p*-aminobenzoate.

Lack of appreciation of naturally-occurring drug antagonists has led to untenable theories of sulphonamide action; though these are now less frequently encountered, methods intended to demonstrate or measure the activities of drugs are still suggested which suffer from the fundamental disability of using media containing indefinite quantities of antagonists. Tissue culture methods have been advocated which, though otherwise of great value, use chicken-embryo extracts or diluted plasma (Osgood and Brownlee, 1938; Osgood, Brownlee and Joski, 1940; King, Henschel and Green, 1939; Jacoby, Medawar and Willmer, 1941) rather than untreated human fluids or solutions of known antagonistic power. Variations in the concentration and metabolism of drug antagonists in hosts of different species are factors to be considered in transferring results from animal experiments to human practice, and their determination a contribution to the assessing of different drug activities in different hosts.

SUMMARY.

A method of assessing *in vivo* activities of drugs by *in vitro* experiments is illustrated by investigations with sulphanilamide. The quantities of the drug necessary for bacteriostasis in the presence of its main antagonist have been found, and the concentration of the antagonist in tissue fluids determined. From this the minimal concentration of the drug which should be necessary for action *in vivo* has been calculated. This concentration is always reached in practice.

I am greatly indebted to Mr. D. E. Hughes for assistance throughout these investigations ; to Dr. M. Stephenson for cultures of *Cl. acetobutylicum* and practical details concerning them ; to Drs. A. Wilson and K. J. G. Milne for collecting blood specimens, and to Dr. V. du Vigneaud for a gift of pure biotin.

REFERENCES.

BECHHOLD, H., AND EHRLICH, P.—(1906) *Hoppe-Seyl. Z.*, **47**, 173.
BLANCHARD, K. C.—(1941) *J. biol. Chem.*, **140**, 919.
DEUTSCH, A., EGGLETON, M. G., AND EGGLETON, P.—(1938) *Biochem. J.*, **32**, 203.
FILDES, P.—(1938) *Brit. J. exp. Path.*, **19**, 239.—(1940) *Ibid.*, **21**, 67.
FLEMING, A.—(1940) *Proc. Roy. Soc. Med.*, **33**, 487.
HARROW, B., MAZUR, A., AND SHERWIN, C. P.—(1933) *J. biol. Chem.*, **102**, 35.
JACOBY, F., MEDAWAR, P. B., AND WILLMER, E. N.—(1941) *Brit. med. J.*, ii, 149.
KING, J. T., HENSCHEL, A. F., AND GREEN, B. S.—(1939) *J. Amer. med. Ass.*, **113**, 1704.
KOCH, R.—(1881) *Mitt. Kaiserl. Gesundheitsamtes*, **1**, 234.
MCILWAIN, H.—(1939) *Brit. J. exp. Path.*, **20**, 330.—(1942) *Ibid.*, **23**, 95.
MACLEOD, C. M.—(1940) *J. exp. Med.*, **72**, 217.
MARTIN, G. J., AND FISHER, C. V.—(1942) *J. biol. Chem.*, **144**, 289.
OSGOOD, E. E., AND BROWNLEE, I. E.—(1938) *J. Amer. med. Ass.*, **110**, 349.
Idem AND JOSKI, J.—(1940) *Amer. J. med. Sci.*, **200**, 596.
OXFORD, A. E., LAMPEN, J. O., AND PETERSON, W. H.—(1940) *Biochem. J.*, **34**, 1588.
PARK, C. R., AND WOOD, W. B., JUN.—(1942) *Bull. Johns Hopk. Hosp.*, **70**, 19.
QUICK, A. J.—(1932) *J. biol. Chem.*, **96**, 83.
ROSE, H. M., AND FOX, C. L.—(1942) *Science*, **95**, 412.
RUBBO, S. D., AND GILLESPIE, J. M.—(1940) *Nature, Lond.*, **146**, 838.—(1942) *Lancet*, i, 36.
SELBIE, F. R.—(1940) *Brit. J. exp. Path.*, **21**, 90.
STRAUSS, E., LOWELL, F. C., AND FINLAND, M.—(1941) *J. clin. Invest.*, **20**, 189.
WOOD, W. B.—(1942) *J. exp. Med.*, **75**, 369.
WOODS, D. D.—(1940) *Brit. J. exp. Path.*, **21**, 74.
WYSS, O., GRUNBAUGH, K. K., AND SCHMELKES, F. C.—(1942) *Proc. Soc. exp. Biol. N.Y.*, **49**, 618.

A STUDY OF THE MITOTIC ACTIVITY OF NORMAL HUMAN BONE MARROW.

J. JAPA.

From the Polish Medical School, and the Institute of Animal Genetics, University of Edinburgh.

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THE mitotic activity in human haemopoietic organs, especially bone marrow, has been reported frequently, but so far only a few systematic analyses have been carried out, and all of these were based on material prepared by methods unsuitable for karyological investigation. The recent introduction of sternal puncture and bone marrow examination into clinical work and the improvement in cytological technique provide an opportunity to undertake such a study. Apart from the purely haematological side of this research there is a general cytological interest too. Bone marrow is the organ which retains the property of embryonic mesenchymal tissue, and its relatively large and isolated young blood cells are specially suited for studying the division and chromosome behaviour of human somatic cells.

At present, clinical examination of bone marrow is limited to recording the proportion of the various marrow elements and the possible presence of pathological cells, while as regards mitotic activity, investigators are satisfied with stating whether the dividing cells are numerous or not. It is expected that a detailed quantitative as well as qualitative analysis of mitosis in normal and abnormal bone marrow will yield interesting data, and may throw light upon the processes of normal and pathological proliferation of blood cells. The aim of the present paper, which is introductory, is to describe a new method and to report upon the mitotic activity of normal bone marrow.

Mitosis in haemopoietic organs was first reported by Fleming (1891). He stated that the proliferation of normal white cells takes place by ordinary mitotic division. This was corroborated by Maximow in 1910 and later by several others. Segerdahl (1935) in her work on sternal puncture gave some quantitative data concerning the mitotic activity of normal bone marrow. She found that the frequency of dividing cells amounts to 4·6 per 1000 myelocytes, 21·8 per 1000 myeloblasts, and 11·7 per 1000 erythroblasts. Her data are only an approximate estimate on account of the technique used. Schwartz (1938), examining bone marrow in cases of megaloblastic anaemia, found mitosis in megaloblasts, and was the first to point out that the cells of bone-marrow tissue are very suitable for studying human chromosomes. Angelini

and Barasciutti (1938) stated that it is possible to identify the different types of marrow cells during division using panchromatic staining. Fieschi (1938) counted and compared the proportion of different mitotic stages amongst young cells of the leuco- and erythroblastic systems. On this basis he draws " karyological " curves and shows that they are different in different haematological diseases.

MATERIAL AND TECHNIQUE.

The samples of bone marrow examined in the present study were taken from three male adults* (marked in the Tables I, II, III), aged between 18 and 35, patients of the Edinburgh Royal Infirmary. Their diseases were negligible from the haematological point of view (hyperacidity of slight degree in one case, spontaneous pneumothorax without any pathological changes in internal organs in another, and no clinical changes at all in the third). The cytological examination of peripheral blood, repeated several times before and after sternal puncture, showed no signs of any affection of the haemopoietic system. The amount of haemoglobin and the red and white cell counts were perfectly normal. The myelograms were within normal limits.

The bone marrow was taken by the usual aspiration method from the manubrium sterni or upper part of the corpus sterni in the quantity of 1–2 c.c. and poured on a watchglass. Isolated particles of bone marrow were put immediately into the aceto-carmine solution (2 g. of carmine added to 100 c.c. of 45 per cent. acetic acid and, after two hours' boiling, filtered). Simultaneously ordinary smear films were prepared using Leishman's or Jenner-Giemsa's panchromatic method for staining. The importance of dealing with isolated particles of bone marrow cannot be over-emphasized, since the films thus obtained are rich in marrow cells and contain a comparatively small proportion of circulating blood elements.

The technique of aceto-carmine preparation is as follows: The particles are kept in stain for at least ten minutes and, at most, 24 hours. The red cells are diluted in this solution and do not obscure the picture. From the solution particles are transferred with a pipette to albuminized slides, and covered with coverglasses. The coverglass is tapped with a needle in order to break up the particle and so obtain isolated cells. The slides are then placed in 70 per cent. alcohol for 24 hours to soak off the coverglasses, passed through absolute alcohol, and mounted in euparal. In the aceto-carmine method the structure of all nuclei is very well preserved, the chromosomes are seen very clearly and it is easy to distinguish the various stages of mitosis. The cytoplasm is almost unstained, and owing to that, the identification of the young cells is very difficult, but after some practice it is possible to discriminate between the cells within certain limits of probability. In these preparations the number of dividing cells was counted per 1000 nucleated cells, and in addition the proportion of four stages of mitosis (pro-, meta-, ana- and telophase) per 100 dividing cells at least was recorded irrespective of their origin.

The ordinary panchromatic staining was employed for the morphological

* The author wishes to express his sincere thanks to Dr. T. R. R. Todd for permission to obtain the material for this study.

determination of the different types of cells in division. This method sometimes gives very good pictures showing cells in mitosis (Plate, Fig. 1), but usually many of the dividing cells are damaged owing to smearing, and unsuitable for a proper indentification. By comparing the aceto-carmine method with this, it was found that the destruction of dividing cells is more or less proportionate in various types of cells and in the various stages of mitosis, so that although the absolute number of divisions was less than in the former, the proportion of four mitotic stages was similar in both preparations.

From panchromatic preparations myelograms were made by counting 1000 cells, and the number of cells in division was recorded per 1000 nucleated cells. In order to find out the proportion of dividing cells in the leuco- and erythroblastic systems 100 dividing cells in all were counted. The frequency of different types of cells in division in the two groups was also recorded.

RESULTS.

All data referring to the mitotic activity are shown in the tables.

The first question is : What proportion of dividing cells are to be found in normal bone marrow ? The data obtained from panchromatically stained smear films and from aceto-carmine preparations are different as was already mentioned, the latter showing a larger proportion. While the number of dividing cells in panchromatic films was found to be about 5 per 1000 nucleated cells, it was three times higher in aceto-carmine preparations made from the same material (Table I). This difference was not due to different admixture of current blood, as the number of staff cells and polymorphs (very easily recognizable on both preparations) was the same, proving that the cell composition of both preparations was alike, and so it must be attributed to the technique. In making the smear preparations for panchromatic staining, a good proportion of dividing cells is damaged or destroyed, hence counts on such smears give too low an estimate.

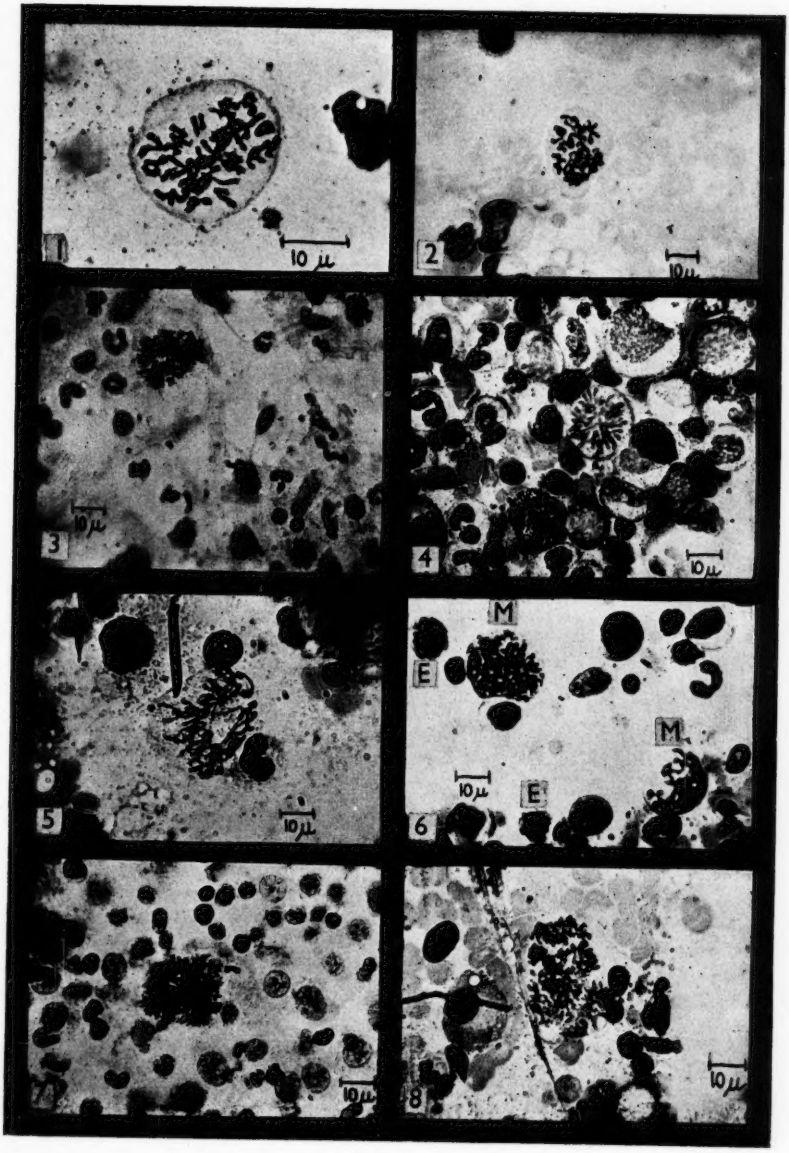
TABLE I.—*The Number of Dividing Cells per* 1000 *Nucleated Cells.*

	I.	II.	III.
In smear panchromatic films	5	6	5
In aceto-carmine preparations	14	16	13

In order to test the similarity between these two distributions χ^2 method has been applied. The obtained value of χ^2 was 0·2371 with two degrees of

DESCRIPTION OF PLATE.

FIG. 1.—Haemocytoblast in metaphase (Leishman's stain).
FIG. 2.—Early erythroblast in division (Leishman's stain).
FIG. 3.—Group of late erythroblasts in division (aceto-carmine).
FIG. 4.—Neutrophilic myelocyte in metaphase and another in early prophase stage (Leishman's stain).
FIG. 5.—Dividing premyelocyte (Leishman's stain).
FIG. 6.—Two dividing myelocytes /*M*/, one damaged, and two dividing late erythroblasts /*E*/ (Leishman's stain).
FIG. 7.—Multinuclear division in giant cell (aceto-carmine).
FIG. 8.—Multinuclear division in giant cell (Leishman's stain).



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freedom, giving P equal to 0·9, showing that in about 90 out of 100 samples the results will be similar to those obtained.

When the proportion of dividing to non-dividing cells was calculated all nucleated cells were considered, since it is impossible to distinguish between the cells which will divide and cells which are already differentiated, i.e., will not go through any more divisions. Hence the data obtained must be considered to be an under-estimate.

The percentage of the four mitotic stages counted on smear films and on aceto-carmine preparations is very similar (Table II). The slight difference in the proportion of prophase and telophase stages is due to the fact that while the prophase is difficult to identify in panchromatic films, the late telophase is difficult to recognize in aceto-carmine preparations. The most frequent stage is metaphase, amounting to 45 per cent. in the aceto-carmine preparations, the prophase accounts for 40 per cent., the anaphase 10 per cent., and telophase 5 per cent. This would indicate that the duration of prophase and metaphase stages is at least four times as long as anaphase and telophase or longer.

TABLE II.—*The Proportion of Four Mitotic Stages.*

Aceto-carmine.	P.	M.	A.	T.	Number of mitoses counted.
I	43·6	43·4	8·8	4·8	500
II	36·0	49·0	10·5	4·5	200
III	40	46	8	6	100
Leishman					
I	30	36	21	13	100
II	35	41	9	15	100
III	35	40	13	12	100

The proportion of dividing leuco- and erythroblasts estimated in panchromatic films is approximately the same in the three cases investigated (Table III), but the data suggest that the ratio of prophases to metaphases is

TABLE III.—*The Proportion of Dividing Leucoblasts to Erythroblasts per 100 Mitoses (Smear Films, Leishman's Stain).*

	Leucoblasts.	Erythroblasts.
I	47	53
II	42	58
III	45	55

apparently different in the two systems (Table IV). However, it is not possible to draw a definite conclusion because the identification of late erythroblasts in prophase is difficult in panchromatically stained films.

The proportion of different young cells participating in division in the leuco- and erythroblastic systems was also determined (Table IV). The data show that in the former the highest proportion of dividing cells is amongst the myelocytes. In the production of white cells, 97 per cent. myelocytes and only 3 per cent. myeloblasts participate. In the erythroblastic system 9 per cent. of dividing cells belong to the early, and 91 per cent. to the late, mostly polychromatophilic, erythroblasts.

TABLE IV.—*The Proportion of Dividing Cells and of Mitotic Stages amongst Different Types of Marrow Cells* (100 *Dividing Cells counted, Leishman's Stain*).

		P.	M.	A.	T.	Total.
Myeloblasts	I	1	—	—	1	2
	II	1	—	—	—	1
	III	1	—	—	—	1
Myelocytes	I	20	14	9	2	45
	II	18	17	3	3	41
	III	22	15	4	3	44
Early erythroblasts	I	—	3	1	1	5
	II	4	2	1	—	7
	III	3	—	1	1	5
Late erythroblasts	I	9	19	11	9	48
	II	12	22	5	12	51
	III	9	25	8	8	50

During the analysis of films giant cells, most probably megakaryocytes, were encountered which contained several independent chromosome complexes (Figs. 7 and 8). The origin and behaviour of such multinuclear mitotic figures is under investigation.

SUMMARY.

1. An analysis of the mitotic activity of human bone marrow, using sternal puncture and the combination of aceto-carmine and panchromatic staining methods, was carried out in three normal individuals.

2. The number of dividing cells in normal bone marrow was found to be 15 per 1000 nucleated cells.

3. The proportion of the respective mitotic stages in normal bone marrow was as follows: 40 prophases, 45 metaphases, 10 anaphases, 5 telophases.

4. The proportion of dividing leucoblasts to erythroblasts per 100 mitoses was about 45 to 55 respectively.

5. In the leucoblastic system 97 per cent. of dividing cells consisted of myelocytes and 3 per cent. of myeloblasts. In the erythroblastic system 91 per cent. of dividing cells were late and 9 per cent. early erythroblasts.

6. In normal bone marrow dividing multinuclear giant cells were seen.

REFERENCES.

ANGELINI, G., AND BARASCIUTTI, A.—(1938) *Arch. Sci. med.*, **66**, 257.
FIESCHI, A.—(1938) *Haematologica*, **19**, 120.
FLEMMING, W.—(1891) *Arch. mikr. Anat.*, **37**, 249.
MAXIMOW, A.—(1910) *Ibid.*, **76**, 1.
SCHWARTZ, E.—(1938) *Amer. J. Cancer*, **33**, 120.
SEGERDAHL, E.—(1935) *Acta med. Scand.*, Supp., **64**, 1.